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# THE PHYSIOLOGY OF FISHES

*Volume II*

# THE PHYSIOLOGY OF FISHES

*Edited by*
MARGARET E. BROWN
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King's College, London

*Volume II*
*BEHAVIOR*

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# CONTENTS

*Page*

Contributors to Volume II . . . . . v

Contents of Volume I . . . . . xi

Chapter I THE NERVOUS SYSTEM . . . . . 1

E. G. Healey

I. Introduction . . . . . 2
II. The Telencephalon . . . . . 3
III. The Diencephalon . . . . . 12
IV. The Mesencephalon . . . . . 16
V. The Metencephalon . . . . . 35
VI. The Medulla Oblongata and Spinal Cord . . . . . 42
VII. The Autonomic Nervous System . . . . . 90
VIII. The Functional Development of the Nervous System . 101
IX. Various Topics Relevant to the Nervous System . . . 103
References . . . . . 104

Chapter II THE SENSE ORGANS . . . . . 121
Part 1. THE EYE . . . . . 121

J. R. Brett

I. Origin and Significance of the Eye . . . . . 121
II. Component Parts of the Visual System . . . . . 123
III. Photoreceptive Functions . . . . . 130
IV. Functional Adaptations . . . . . 144
V. Synopsis . . . . . 147
References . . . . . 150

Part 2. THE ACOUSTICO-LATERALIS SYSTEM . . . . . 155

O. Lowenstein

I. Introduction . . . . . 155
II. The Lateral Line . . . . . 156
III. The Ampullae of Lorenzini . . . . . 164
IV. The Labyrinth . . . . . 168
References . . . . . 182

Part 3. OLFACTORY AND GUSTATORY SENSES OF FISHES . . 187

Arthur D. Hasler

I. Introduction . . . . . 187
II. Definition and Theory . . . . . 187
III. Anatomy of the Olfactory Organs of Fishes . . . . . 188
IV. Impulses from the Olfactory Tissue to the Brain . . 193

*Page*

V. Taste and the Common Chemical Sense . . . 198
VI. Olfaction in Fishes . . . . . . . . 199
VII. Orientation to the Parent Stream as It Relates to Odor Recognition . . . 203
VIII. Concluding Comment . . . 206
References . . . 207

Chapter III BEHAVIOR . . . . . . . 211
Part 1. CONDITIONED RESPONSES . . . 211
H. O. Bull

I The Nature of the Conditioned Response . . . 211
II. Experimental Methods Used in the Formation of Conditioned Responses in Fishes . 216
III. Sensory Investigations by Conditioned Response Techniques . . . . . . . . 218
IV. The Relation of the Laboratory Study of Conditioned Responses to Behavior in Normal Environments, and Its Value to Fishery Investigations . . . 223
References . . . . . . . . 224

Part 2. THE ETHOLOGICAL ANALYSIS OF FISH BEHAVIOR 229
G. P. Baerends

I. Introduction . . . . . . . . . . 229
II. Coordinating Mechanisms . . . . 231
III. Releasing Mechanisms . . . . . . . . . . . . 252
IV The Interaction between Internal and External Factors 258
V. Ritualization . . . . . . . . . . . 263
References . . . . . . . . . . . 265

Part 3. REPRODUCTIVE AND PARENTAL BEHAVIOR . . . 271
L. R. Aronson

I Methodology . . . . . . 272
II Ecological Relationships . . . . . . . . 273
III Sensory Systems in Sexual and Parental Behavior 281
IV Endocrine Mechanisms . 283
V Neural Mechanisms . 288
VI Motor Processes . . . 290
References . 296

Chapter IV THE SWIMBLADDER . 305
F. R. Harden Jones

I Introduction . . . 305
II Hydrostatic Function . . . . . . . . 307
III The Respiratory Function of the Swimbladder 313
IV The Sensory Function of the Swimbladder 313
V Sound Production by the Swimbladder 315

Page

VI. The Swimbladder and the Ecology of the Teleostean Fishes . . . 316
References . . . 318

Chapter V ELECTRIC ORGANS . . . 323
R. D. Keynes

I. The Discovery of Electric Fishes . . . 323
II. The Anatomy of the Electric Organ . . . 324
III. The Origin and Development of Electric Organs . . . 328
IV. The Characteristics of the Electric Discharge . . . 329
V. The Discharge of a Single Electroplate . . . 332
VI. The Nervous Control of the Electric Organ . . . 336
VII. The Biological Function of the Electric Organ . . . 338
VIII. The Immunity of Electric Fishes to Their Own Discharge 340
References . . . 340

Chapter VI THE LUMINOUS ORGANS OF FISHES . . . 345
E. Newton Harvey

I. Historical Introduction . . . 345
II. Distribution of Luminescence . . . 348
III. Types of Luminescence . . . 348
IV. Nerve and Hormone Control . . . 362
V. Use of the Light . . . 363
References . . . 364

Chapter VII THE PIGMENTS OF FISHES . . . 367
D. L. Fox

I. Introduction . . . 367
II. Flavines . . . 368
III. Pterins . . . 369
IV. Melanins . . . 370
V. Carotenoids . . . 371
References . . . 383

Chapter VIII COLOR CHANGES . . . 387
J. M. Odiorne

I. Introduction . . . 387
II. Physiological Color Changes . . . 388
III. Morphological Color Changes . . . 398
IV. Significance . . . 401
References . . . 401

Chapter IX WATER QUALITY REQUIREMENTS OF FISHES AND EFFECTS OF TOXIC SUBSTANCES . . . 403
Peter Doudoroff

I. Introductory Discussion . . . 403

Page
II. Temperature . . . . . . . . . . . . . . . . . 406
III. Salinity and Osmotic Pressure . . . . . . . . . . 409
IV. pH and Dissolved Carbon Dioxide . . . . . . . . 411
V. Dissolved Oxygen and Nitrogen . . . . . . . . . 413
VI. Suspended Solids (Turbidity) . . . . . . . . . . 416
VII. Miscellaneous Toxic Substances . . . . . . . . . 417
References . . . . . . . . . . . . . . . . . 427

Chapter X PHYSIOLOGICAL GENETICS OF FISHES . . . . . . . . 431
Myron Gordon

I. Introduction . . . . . . . . . . . . . . . . . 432
II. Sex Determination . . . . . . . . . . . . . . . 433
III. Effects of Pigment Cell Genes . . . . . . . . . . 447
IV. Genes Influencing Atypical Pigment Cell Growth . . 453
V. Eye Characteristics . . . . . . . . . . . . . . 464
VI. Meristic Traits . . . . . . . . . . . . . . . . 468
VII. Transplantation, Resistance, Serology . . . . . . . 473
VIII. Susceptibility to Thyroid Tumors . . . . . . . . . 478
IX. Isolating Mechanisms and Speciation . . . . . . . 486
X. Summary . . . . . . . . . . . . . . . . . . 492
References . . . . . . . . . . . . . . . . . 493

Author Index . . . . . . . . . . . . . . . . . . . . 503

Subject Index . . . . . . . . . . . . . . . . . . . . 515

# Contents of Volume I

Respiration

I. The Aquatic Respiration of Fish
F. E. J. FRY

II. Air Breathing
G. S. CARTER

The Cardiovascular System
J. C. MOTT

The Alimentary Canal and Digestion
E. J. W. BARRINGTON

Excretion and Osmoregulation
VIRGINIA SAFFORD BLACK

The Skin and Scales
JOHN VAN OOSTEN

Endocrine Organs
WILLIAM S. HOAR

The Gonads and Reproduction
WILLIAM S. HOAR

Early Development and Hatching
SYDNEY SMITH

Experimental Studies on Growth
M. E. BROWN

The Biochemical Composition of Fish
R. M. LOVE

## Chapter I

# THE NERVOUS SYSTEM

E. G. Healey

Page

I. Introduction . . . . . . . . . . . . . . . . . . . . . . . 2
II. The Telencephalon . . . . . . . . . . . . . . . . . . . 3
  A. Anatomy . . . . . . . . . . . . . . . . . . . . . . 3
  B. The Telencephalon and the Olfactory Sense . . . . . . . . 4
  C. Experimental Work Connected with Normal Swimming, Balance, and Feeding . . . . . . . . . . . . . . . . . . . . 6
    1. Extirpation of the Telencephalon . . . . . . . . . . 6
    2. Electrical and Chemical Stimulation of the Telencephalon . . . 7
  D. The Telencephalon and Association Centers . . . . . . . . 7
  E. The Telencephalon and Other Aspects of Behavior . . . . . . 8
III. The Diencephalon . . . . . . . . . . . . . . . . . . . 12
  A. Anatomy . . . . . . . . . . . . . . . . . . . . . . 12
  B. Experimental Work Involving Extirpation of the Diencephalon . . 13
  C. Experimental Work Associated with Color Change and Light Perception 13
IV. The Mesencephalon . . . . . . . . . . . . . . . . . . . 16
  A. Anatomy . . . . . . . . . . . . . . . . . . . . . . 16
  B. The Effects of Lesions and Removal of Parts of the Midbrain upon Posture and Movement . . . . . . . . . . . . . . . . 16
  C. Motor Effects Following Stimulation of the Midbrain . . . . . 18
  D. Relations of the Optic Tectum to Vision and to Motor Activity . . 20
  E. Electrical Signs of Activity in the Optic Tectum . . . . . . 32
  F. The Relations of the Optic Tectum to Learning and Conditioned Responses . . . . . . . . . . . . . . . . . . . . . 34
V. The Metencephalon . . . . . . . . . . . . . . . . . . . 35
  A. Anatomy . . . . . . . . . . . . . . . . . . . . . . 35
  B. Early Investigations on the Physiology of the Metencephalon in Fishes 36
  C. Physiological Investigations on the Elasmobranch Metencephalon . 36
  D. Physiological Investigations on the Teleost Metencephalon . . . 38
  E. Anatomical Indications of the Possible Function of the Corpus Cerebelli 40
  F. Physiological Investigations on the Role of the Metencephalon in the Formation of Associations . . . . . . . . . . . . . . 40
VI. The Medulla Oblongata and Spinal Cord . . . . . . . . . . 42
  A. The Medulla Oblongata . . . . . . . . . . . . . . . . 42
    1. Anatomical Relations of the Medulla Oblongata . . . . . . 42
    2. Physiological Investigations on the Medulla Oblongata . . . 43
      a. Equilibrium and the acoustico-lateralis system . . . . . 43
      b. Respiratory movements . . . . . . . . . . . . . . 43
      c. The control of chromatophores . . . . . . . . . . . 46
  B. The Spinal Cord . . . . . . . . . . . . . . . . . . . 46
    1. Anatomical Relations of the Spinal Cord . . . . . . . . 46
    2. Segmental Innervation of the Skin, Muscles, and Chromatophores 46

3. Aspects of Excitability and Conduction in the Spinal Cord . . . 49
    a. The excitability of the spinal cord . . . . . . . . . . 49
    b. The effects of some poisons upon the spinal cord . . . . 49
    c. The spinal cord as a conducting organ . . . . . . . . . 50
4. Regeneration in the Spinal Cord . . . . . . . . . . . . . 51
C. The Part Played by the Medulla Oblongata and the Spinal Cord in the Nervous Control of Muscular Movements and Locomotion . . 53
1. Introduction . . . . . . . . . . . . . . . . . 53
2. Unisegmental Reflexes . . . . . . . . . . . . . . 53
3. Plurisegmental Reflexes . . . . . . . . . . . . . 54
    a. Experiments on elasmobranchs . . . . . . . . . . . 54
    b. Experiments on teleosts . . . . . . . . . . . . . 57
4. The Conduction of Locomotory Movements along the Body . . 66
5. The Spontaneity of Action of the Spinal Cord and the Initiation of Locomotory Movements . . . . . . . . . . . . . 72
    a. Theories . . . . . . . . . . . . . . . . . 72
    b. Some investigations on cyclostomes and elasmobranchs . . . 72
    c. Some investigations on teleosts . . . . . . . . . . 74
    d. The case for an automatic rhythmical mechanism, independent of peripheral stimulation . . . . . . . . . . . 76
    e. The case for the dependence of locomotory activities upon peripheral stimulation . . . . . . . . . . . . . 79
6. The Coordination of Fin Movements . . . . . . . . . . 86
VII. The Autonomic Nervous System . . . . . . . . . . . . 90
A. Anatomy . . . . . . . . . . . . . . . . . . . 90
B. Functions Associated with the Alimentary Canal . . . . . . 91
C. Functions Associated with the Swim-Bladder . . . . . . . 92
D. Functions Associated with the Urinogenital System . . . . . 93
E. Functions Associated with the Heart and Vascular System . . . 93
F. Functions Associated with the Iris . . . . . . . . . . 97
G. Functions Associated with the Control of Chromatophores . . . 97
VIII. The Functional Development of the Nervous System . . . . . 101
A. Introduction . . . . . . . . . . . . . . . . . 101
B. Cyclostomes . . . . . . . . . . . . . . . . . 102
C. Elasmobranchs . . . . . . . . . . . . . . . . 102
D. Teleosts . . . . . . . . . . . . . . . . . . 103
IX. Various Topics Relevant to the Nervous System . . . . . . 103
A. The Cerebrospinal Fluid . . . . . . . . . . . . . 103
B. Acetylcholine . . . . . . . . . . . . . . . . . 104
C. "Moods," "Hypnosis," and General Inhibition . . . . . . 104
References . . . . . . . . . . . . . . . . . . 104

## I. INTRODUCTION

The physiology of the nervous system of fishes did not receive much attention before the third decade of the last century. From that time publications appeared in increasing numbers so that today the literature, while still very restricted, has reached proportions which enable some guarded generalizations to be made. The bibliography at the end of this chapter is not exhaustive but further references to the various topics may be obtained from the publications listed in it.

Our knowledge of the anatomy of the fish nervous system has, in general, far outstripped our physiological knowledge, as is common enough in other fields. It is, however, possible to find cases where experimental work has been indulged in to little profit through lack of adequate knowledge of the anatomical structures concerned. The physiological work which has been done is largely restricted to elasmobranchs and teleosts. Little is known about the physiology of the nervous system in cyclostomes and nothing about it in Dipnoi. So far as some topics in this chapter are concerned, the total amount of work which has been done is so scanty that elasmobranchs and teleosts will be considered together, but where the amount of available information justifies it, they will be treated separately.

Reference may be made to Kappers *et al.* (1936) for details of anatomy. Regarding physiological matters, the reader may refer for a comparative treatment to von Buddenbrock (1953) and, for work on mammals, to a text such as that by Fulton (1949). The only recent work devoted solely to the physiology of the nervous system of fishes is the valuable review by ten Cate (1935). Steiner's book (1888) must be regarded as a landmark in the development of the subject although today parts of it are only of historical interest. Finally, reference to the fine structure of the nervous system in fishes and to aspects of more general nerve physiology may be found in Carlson (1904), Graham and O'Leary (1941), Bodian (1938, 1951), Weber (1952), and the Cold Spring Harbor Symposium on the neurone (1952) as well as elsewhere in the present text.

The fishes vary greatly with regard to the use which they make of their various sense organs and the control which they exercise over their various effectors. The importance of one or another sensory or effector system can be correlated in many cases with the relative size of the corresponding regions in the central nervous system (Herrick, 1922; Bhimachar, 1937; Evans, 1952; Kappers *et al.*, 1936). Further, there are great differences among fishes regarding their behavior and, one may therefore suppose, regarding corresponding details of internal brain anatomy. In short, while they may be permissible in some cases, generalizations about the physiology of the nervous system of fishes, or even of the separate classes of fishes, must be regarded with reserve until a much greater number of types has been investigated.

## II. THE TELENCEPHALON

### A. Anatomy

Anatomical investigations show that the telecephalon or forebrain of fishes stands in close relation to the olfactory organ. While this is the most obvious anatomical feature, there are also ascending and descending fibers connecting it to other parts of the central nervous system. Ascending fibers come from the hypothalamus and are thought to be visceral and, in particular, gustatory, since Herrick (1905) showed that taste fibers end in the lobi inferiores. The descending fibers pass to the hypothalamus, the ventral thalamus, and the epithalamus where they make connection with fibers of other ascending and descending systems (Kappers *et al.*, 1936; Herrick, 1922). It is thus evident that although the telencephalon in fishes is largely associated with the olfactory sense, it appears also to contain correlation centers. However, the cerebrum with a cortex, as we know it in higher vertebrates, does not exist in the fishes. In them the primitive cerebrum contains relatively well-developed basal ganglia or corpora striata but it is comparatively a very rudimentary structure and nothing is yet known with certainty of its functions although, as will be seen, there are now some indications.

It is worth stressing at this point that the relative size and the gross shape and structure of the cerebrum vary considerably throughout the fishes. This can be seen even when nearly related species are compared. When the comparison is made between the cyclostomes, elasmobranchs, teleosts, and dipnoans the differences are clearly very great (Fig. 1). Their physiological significance is, however, not known.

Extirpation and electrical and chemical stimulation have been the chief means of attacking the problem of the functions of the telencephalon of fishes but, apart from the rather obvious connection with the sense of smell, the outcome of most of the investigations has been a mere list of functions which it does not have. Positive qualities have, generally speaking, escaped attention until the last few years.

### B. The Telencephalon and the Olfactory Sense

Steiner (1888) observed that the teleost *Squalius cephalus* no longer detects food with its nose but only with its eyes after removal of the forebrain. Similarly the elasmobranch *Scyliorhinus*, which relies to a very great extent upon its olfactory sense, is at a serious disadvantage after

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FIG. 1. *a* and *c*: Dorsal views of the generalized brains of an elasmobranch (based upon *Scyliorhinus*) and of a teleost (based upon *Salmo*). *b* and *d*: Schematic longitudinal sections through *a* and *c*. In the forebrain the sections pass through the ventricle of one side.

*bas. g.* basal ganglion; *corp. cereb.* corpus cerebelli; *di.* diencephalon; *fore.* forebrain; *hyp.* hypophysis; *l.aur.cereb.* left auriculus cerebelli; *med.obl.* medulla oblongata; *mes.* mesencephalon; *met.* metencephalon; *myel.* myelencephalon; *olf.b.* olfactory bulb; *olf.tr.* olfactory tract; *op.* optic nerve and tract; *pin.* pineal; *r. and l corp. bigem.* right and left corpora bigemina of the optic tectum; *sac. vasc.* saccus vasculosus; *tel.* telencephalon; *valv. cereb.* valvula cerebelli.

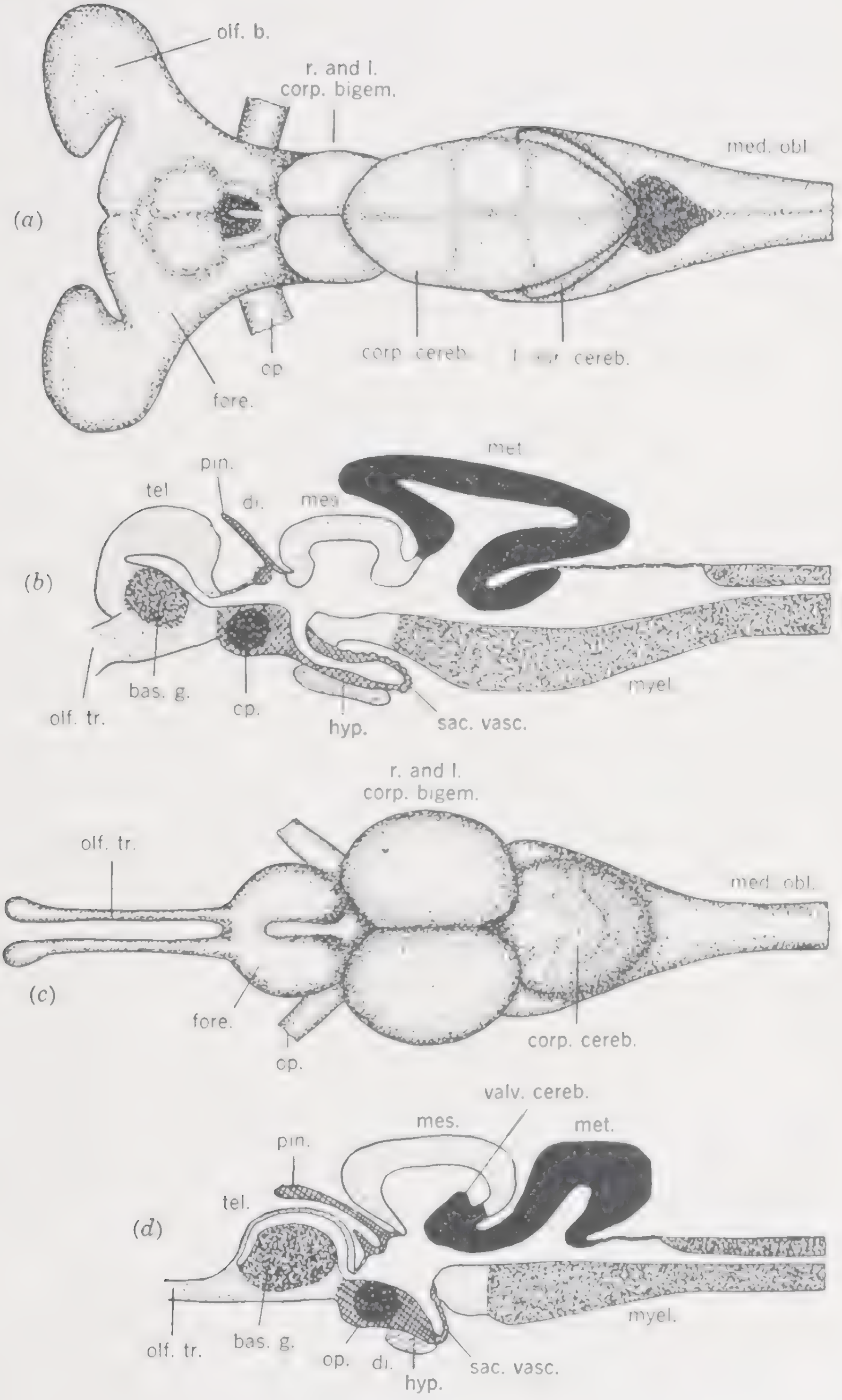
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FIGURE 1

this operation and can no longer search for its food effectively. Nagel (1894) and Sheldon (1909) drew attention to the possibility of confusing the sense of smell with that of taste in fish and this was taken into account by Parker (1910, 1911) in testing the olfactory powers of *Ameiurus* and *Fundulus*. Strieck (1925) demonstrated clearly that smell and taste are separate senses in the minnow (*Phoxinus*) and are associated with different parts of the central nervous system. He trained blinded minnows to respond to substances with a taste as distinct from substances with a smell. After removal of the forebrain the response to the former was unchanged but substances were no longer detected by smell.

## C. Experimental Work Concerned with Normal Swimming, Balance, and Feeding

### 1. EXTIRPATION OF THE TELENCEPHALON

According to ten Cate (1935), Desmoulins (1825) was the first to extirpate the telencephalon in various teleosts. After the operation he was unable to see any difference in the movements or balance of the animals. His results were confirmed by Magendie (1838), Flourens (1842), Baudelot (1864), Renzi (1864), and Vulpian (1866, 1886). Ferrier (1876) claimed that after the operation his fish, although normal in every other way, tended to move continuously, only stopping when they reached the wall of their tank or when they were exhausted. While most of the fishes from which the forebrains had been removed by earlier workers very soon died, Steiner (1888) was able to keep *Squalius cephalus* alive indefinitely after the operation. Vision, movements, balance, and feeding habits were unchanged and the fish could not be distinguished from a normal animal. Polimanti (1912b) reported disturbances of balance and vision after removing the forebrain in *Crenilabrus* but it seems probable that other parts of the brain were accidentally damaged (ten Cate, 1935). Reisinger (1915) found that removal of the forebrain had no visible effect upon *Perca fluviatilis* and Meader (1939) found that *Holocentrus* likewise appeared to swim normally after the same operation.

Similar experiments have been made on elasmobranchs. Steiner (1888) removed the forebrains from *Scyliorhinus catulus* and *S. canicula* and reported that vision, swimming, and balance remained normal but that there was a tendency for the animals to lie on the bottom and to stop their customary search for food. Since these fish are very dependent upon their olfactory sense for orientation and for feeding, Steiner supposed that the loss of this sense, rather than the loss of the forebrain in any other capacity, was responsible. Loeb (1891), Bethe (1899), and Polimanti

(1911, 1912a) similarly reported no disturbance of movement or balance after removal of the forebrain in various elasmobranchs. According to Springer (1928) section of the olfactory tracts in *Mustelus canis* and *Squalus acanthias* had no effect upon respiratory movements but the latter became convulsive after transection of the forebrain. The paper gives little detail and it is difficult to assess this statement. Rizzolo (1929b) made careful operations on the smooth dogfish (*Galeus canis*) and found that after removal of the forebrain there was no disturbance of equilibrium: if the animal was placed on its back at the surface of the water it righted itself immediately; it swam normally and at the bottom of the aquarium it settled dorsal side up.

### 2. Electrical and Chemical Stimulation of the Telencephalon

Ferrier (1876) applied electrical stimulation to the forebrain of elasmobranchs and teleosts and described fin and eye muscle movements resulting from it. However, there seems to be no doubt that these were caused by the spread of too strong a stimulating current to the mesencephalon and other regions of the brain. Similar claims were made by Polimanti (1912b), following electrical stimulation experiments on teleosts, but he (1911, 1912a) found no indication of either stimulation or loss of function when he injected solutions of curare or cocaine into the forebrain of *Torpedo* and *Scyliorhinus*. Chauchard and Chauchard (1927a, b) stimulated the forebrain of the teleosts *Mugil auratus* and *Trigla gurnardus* with condenser discharges and also produced indefinite movements of the body which they rightly ascribed to spreading current. In the rays *Dasyatis pastinaca* and *Myliobatis aquila* they were able to carry out the stimulation of the forebrain more easily and here they found no effect upon the body musculature. Springer (1928) similarly obtained no visible response to electrical stimulation of the forebrain in *Mustelus* and *Squalus*.

All these experiments on teleosts and elasmobranchs have thus led to results which agree fully: they indicate that neither extirpation nor stimulation of the telencephalon leads to any changes in vision, locomotion, balance, or significant behavior. However, from experiments to be described later, it will be seen that this conclusion, in so far as behavior is concerned, is no longer acceptable.

## D. The Telencephalon and Association Centers

It has been established for many years that fishes readily form associations and can be trained to respond to a variety of stimuli and situations (Herter, 1953). Thus Froloff (1925, 1928) showed that fishes can differentiate between various stimuli and form conditioned reflexes which can then be extinguished (see Chap. III: Part I). Nolte (1933) removed the forebrain in the minnow (*Phoxinus*) and stickleback (*Gasterosteus*). Like the earlier workers, he noted no difference in the general behavior of the fish. Further, he found that partial or total extirpation did not affect the ability of the fish to form associations. Thus they could still be trained to colored papers and to spectral colors and minnows which had been trained to distinguish forms still did so after the operation. These significant experiments show that the formation of associations in teleosts takes place in parts of the brain other than the telencephalon. Scharrer (1928) came to a similar conclusion with regard to light stimuli (p. 14).

### E. The Telencephalon and Other Aspects of Behavior

The investigations on the telencephalon which have been mentioned point only to a connection with the olfactory sense but accumulating experimental evidence indicates that it may play a part in the control of higher functions. Thus Kumakura (1927) observed the behavior of small groups of goldfish (*Carassius auratus*) after removal of the forebrain. Fifteen minutes after the operation the animals were making normal swimming movements, although they tended to lie motionless on the bottom. For some time they only swam singly but between the fifth and the fourteenth day they recovered the ability to swim in shoals and in formation like normal fish.

Janzen (1933) found no disturbances of locomotion or balance after extirpation of the forebrain in the goldfish (*Carassius auratus*). After removal of the pectoral and pelvic fins goldfish without forebrains could still swim and maintain their balance. Even when all the fins were removed the position of the body was normal when the animal was at rest. Such observations agree with those of the other workers already cited, but Janzen went further and distinguished between "initiative" and "spontaneity." By "initiative" he meant the ability of the animal to react to a definite external factor by active body movements in a particular and not purely reflex manner, whether the factor concerned had just appeared or had been for a long time in the animal's surroundings. By "spontaneity" he meant the ability to carry out, alter, or inhibit an active body movement without reference to any external factor and without external cause. According to Janzen, after removal of the forebrain in the goldfish spontaneity is scarcely altered but initiative is lost. He gave the following experimental results and his interpretation:

(a) The taking of food is not affected by the operation because it depends upon predominantly reflex behavior.

(b) In the intact fish the rate of the opercular movements is continually changing but after removal of the forebrain these movements are much more even. The fish no longer responds to the outside influences which are the main cause of variations in opercular movements in the normal animal.

(c) For a similar reason the frequency of the eye movements carried out by a normal fish varies considerably but becomes much more nearly constant after removal of the forebrain.

(d) After the operation there is a change in the optomotor response of the fish to the rotation of a striped drum around it. The normal fish occasionally carries out flight reactions and ceases for a time to follow the stripes but after removal of the forebrain the animal appears to be uncompromisingly bound to them. It has lost its initiative so that the reaction takes on much more the character of a reflex. If the forebrain is removed on one side only the rigid reflex character of the response is less extreme.

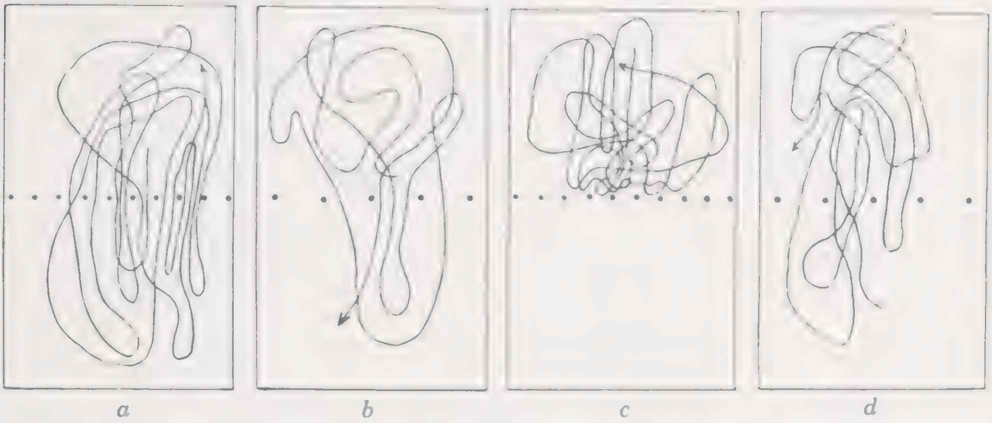

FIG. 2. *a, b:* Path followed in 3 minutes by an unoperated goldfish (*Carassius auratus*) 7 cm. long, using a grid with a space between the vertical rods of 1.8 and 4.0 cm., respectively. *c, d:* Path followed under the same conditions by two fish after removal of the forebrain (from Janzen, 1933).

(e) When placed in an aquarium separated into two portions by a vertical grid of narrow rods the normal fish does not regard this as an obstruction but swims readily to and fro between the rods; in fact, a narrow grid seems to attract the fish to swim through it. This kind of initiative disappears after removal of the forebrain and the animal no longer swims at all through a narrow grid but avoids the rods and so remains permanently on one side (Fig. 2). Again, the animal in which only one half of the forebrain has been removed seems to be intermediate in that some, but not all, of its initiative is lost.

(f) Janzen was able to distinguish between those fish which, when presented with the alternatives of a dark or light colored chamber, tend to choose the light and those which tend to choose the dark. Intermediate types also exist. In order to explain the various types of response Janzen assumed that two intracentral factors were concerned: one, a tendency to seek out a definite level of brightness; the other, a persistence-tendency as a result of which the fish tends to remain in whatever degree of brightness it may find itself, i.e. if it has been on a light background it will tend, when given alternatives, to choose light rather than dark, and so on. After removal of the forebrain the first tendency is lost and only the persistence-tendency remains.

(g) Both normal fish and those without the forebrain can learn to associate a color with feeding but training takes a little longer in the operated animals and they are never quite so certain in making their choice as are the intact animals.

(h) In general, intact fish at first show a certain caution toward new and unfamiliar conditions. After removal of the forebrain this caution disappears and the fish behave much more uniformly toward stimuli and react generally in a much more stereotyped manner.

Janzen's observations have been cited at some length because of their importance in indicating that the telencephalon exerts a control over the other parts of the fish brain. They have been confirmed and extended by later workers. Thus Hosch (1936), working on *Phoxinus* and *Gobio fluviatilis*, also found that removal of the forebrain resulted in a loss of initiative, in Janzen's meaning of the term, while spontaneity was only a little reduced. Because of this, *Phoxinus* and, particularly, *Gobio* are less readily stimulated after the operation and tend to move much less than normal fish and to lie on the bottom. At the same time, a stimulus, once it has become effective, results in flight movements of unusual violence because the reaction to the stimulus is unmodified. Hosch confirmed Janzen's observations regarding eye movements, choice of light or dark backgrounds (*Gobio*), and caution when confronted with unfamiliar situations. His results with the optomotor response in *Phoxinus* agreed with those of Janzen when the whole forebrain was removed but he failed to note any effect when one side only or the anterior halves only were taken away. While Hosch was of the opinion that the swimming of minnows (*Phoxinus*) in shoals does not depend upon the presence of an intact forebrain (see Kumakura, 1927), he did think that the latter plays some part in the formation of groups of individuals in *Gobio*. Similarly Noble in 1936 and 1937 and Wiebalck in 1937 reported differences in schooling and breeding behavior after removal of the forebrain (quoted in Meader, 1939).

Berwein (1941) considered the possibility that the sense of smell, rather than any other special function associated with the forebrain, might be playing a part in some aspects of the social life of minnows. She observed groups of 4–12 fish confined in large tanks and chosen from individuals of the same size so that they formed a small shoal. As well as using normal fish she used groups of which every member was deprived of its bulbus olfactorius and others in which the entire forebrain had been removed from each member. When a small minnow (1.3–1.6 cm.) was put into the tank with a well-formed shoal of larger (8–9 cm.) minnows, it was hunted and eaten. If the newcomer was bigger than the members of the shoal, it too was immediately or very soon driven away but was later accepted into the shoal. Shoals composed of normal fish or of fish deprived of the bulbus olfactorius or of the entire forebrain all reacted in this way, so that the olfactory sense was evidently not concerned in this behavior. No distinction could be observed between normal fish and those without the bulbus but the groups without the entire forebrain showed a slight tendency to delay the initial rejection of the newcomer. The difference between the fish without forebrain and the other two groups was much more striking with regard to the acceptance of the newcomer. The minnows without forebrains took it relatively quickly into the shoal, thus indicating "disinhibition," while the normal fish and those with only the bulbus removed took a very much longer time.

This "disinhibition," resulting from forebrain removal, was shown in another way: when a new group of minnows was put into a tank the animals were disturbed at first and did not form a shoal or feed. Berwein assumed that they were accustomed to their surroundings when they approached their food without hesitation. For normal fish the average time which the fish spent in the tank before this happened was 18 days; for fish without bulbus, 13 days; for fish without forebrain, 7 days.

In these aspects of behavior concerned with the acceptance of newcomers and hesitation in unfamiliar surroundings the sense of smell appeared to be playing no part and the changes found after removal of the forebrain were attributed by Berwein to other functions associated with it, in agreement with the conclusions of Janzen and Hosch.

In another series of observations Berwein found that each minnow in a group of four fish moved more than it did when alone. After removal of the forebrain the animals became less active but they were still sensitive to the differences between solitary and shoal life. Berwein therefore thought that her minnows did not show either a lack of sensitivity to stimuli or inhibition. In order to try to find out why they showed a tendency to move less actively after removal of the forebrain, Berwein compared the behavior under similar conditions of a shoal of minnows in which only the bulbus was removed. Such fish were still less active than those without a forebrain and Berwein suggested that, in this particular situation, the stimulation of one animal by another does in fact depend partly upon the olfactory sense; that fish without the bulbus, having lost this sense, suffer from the lack of it more than the fish without the forebrain because the latter quickly become accustomed to new conditions and probably also to the loss of smell. She thus attempted to explain a situation which at first sight appeared to be anomalous and to show that it could be reconciled with the concepts put forward by Janzen.

Few experiments have so far been performed in order to find out whether the telencephalon of fishes has functions other than those associated with the sense of smell but it already seems certain that higher functions may be assigned to it. As Meader (1939) has pointed out, the fact that the fishes differ greatly in their behavior and the fact that the anatomy of the telencephalon shows very considerable variation both point to the possibility of as great a variation in the physiology of this region and to the necessity for studying many different species before we can arrive at reliable conclusions.

## III. THE DIENCEPHALON

### A. Anatomy

The diencephalon in fishes (Fig. 1) is a small but highly complex structure which is partly covered from behind by the well-developed mesencephalon. Dorsally the pineal organ or epiphysis and the parapineal organ form gland-like structures. The thalamic region, particularly dorsally, is only slightly developed but the ventral-lying hypothalamus is relatively large. These more ventral portions are connected by incoming and outgoing fibers to many parts of the brain and clearly form, on anatomical grounds, a correlation center for olfactory, gustatory, and other types of sensory impulses. Also associated with this region are nuclei belonging to the autonomic nervous system. Various structures grow out from the ventral region of the hypothalamus. The lobi inferiores form paired structures behind which lie the paired corpora mammillaria. The infundibulum grows down to form the hypophysis and behind it is the saccus vasculosus, a structure particularly well developed in deep sea fishes and thought to be a pressure receptor by Dammerman (1910). Cytological evidence, however, is rather in favor of a secretory function, according to van de Kamer and Verhagen (1954). Another unpaired structure, the recessus posterior, may be connected with the resorptive function of the ependymal layer of the brain, according to these same workers (see also Addens, 1946).

Little is known of the physiology of the diencephalon in fishes other than the results of a few experiments on its removal and on functions associated with color change and with light perception.

### B. Experimental Work Involving Extirpation of the Diencephalon

Steiner (1888) cut through the brain of *Scyliorhinus canicula* at the posterior level of the diencephalon and removed it together with the telencephalon. The inevitable destruction of the optic nerve blinded the animal but, it appeared otherwise to be unaffected, swimming in a normal fashion with no disturbance of its movements. However, after some time the fish came to rest in a corner or against a wall of the tank and only swam when it was stimulated. Steiner believed that the fish is normally excited to swim through peripheral stimulation by the moving water and that some of the excitations from the periphery reach the diencephalon. Since this had been removed, the nervous apparatus concerned with the initiation of swimming had been put out of action although there was no abnormality in the locomotory control itself.

Springer (1928) cut through the thalamus of *Mustelus canis* and *Squalus acanthias* and also stimulated it electrically. She reported that these operations resulted in irregular respiratory movements.

### C. Experimental Work Associated with Color Change and Light Perception

Von Frisch (1911a, b) stimulated the central nervous system of the minnow (*Phoxinus*) electrically and concluded that there was a "paling center" in the medulla whose activity was responsible for melanophore aggregation. Spinal section slightly posterior to the medulla resulted in the separation from this paling center of all the melanophores on the surface of the body so that their pigment became dispersed and the animal went dark. Von Frisch made cuts further forward and found that he only ceased to get darkening when the section was at the anterior end of the medulla. If the cut included both medulla and cerebellum there was still darkening but if it was made between the medulla and the mesencephalon, or still further forward in the mesencephalon itself, the fish often became very pale. Removal of the cerebellum had no effect upon these results. They indicated that, in addition to the medullary paling center, there was a possible darkening center, inhibiting the paling center, in the anterior region of the brain. Unipolar electrical stimulation of the forebrain was followed by darkening of the body while stimulation of the midbrain was without effect. Since, however, the telencephalon, as shown by its extirpation, has no effect on the color change of the minnow, the darkening was probably the result of stimulating the diencephalon and the darkening center was accordingly thought to be in this region. (For a further discussion of this subject see p. 97.)

Apart from color changes in response to illuminated backgrounds, fish have often been shown to become paler in darkness and to respond directly to illumination by some degree of melanophore dispersion. This is particularly clear if the animal is blinded so that the background tint plays no part. In some fishes the receptors concerned in this reaction have not been found. Thus Abramowitz (1936b) and Wykes (1938) described the reaction in the catfish (*Ameiurus nebulosus*) but Wykes could find no evidence for a particular photoreceptive region or for the direct stimulation of the melanophores by light. In some fishes the response to light is certainly local while in others it depends upon the illumination of a restricted part of the body. Thus von Frisch found in the minnow that the blinded animal only darkened when light fell upon the top of the head on a spot which was more or less free from melanophores and lay above the epiphysis. Local illumination of this region resulted in darkening of the whole body while when it was shaded the animal became pale. After removal of the overlying tissues illumination of the brain alone would produce the response. Von Frisch concluded that the minnow has a functional parietal organ but was not able to determine the exact position of the sensory region. He suggested that the light-sensitive cells might be looked for in the epithelial lining of the ventricle of the diencephalon. In further work on the minnow Scharrer (1928) showed that the blinded fish can be trained to snap for food when the light intensity is increased and that this sensitivity to light is localized in the diencephalon.

Young (1935) investigated the epiphysial eye in the roof of the diencephalon of *Lampetra fluviatilis*. He found that a bright spot of light directed upon the pineal region of a stationary ammocoete larva usually initiates movement after many seconds. However, these movements can be elicited after the pineal and parapineal organs and the eyes have been removed. There must therefore be other receptors of light intensity in the diencephalon. The pineal region in *Lampetra* is also associated by nervous paths with the hypophysis. The animal shows marked rhythms of color change brought about by pituitary hormones and these are disturbed or abolished by destruction of the pineal region.

Breder and Rasquin (1947, 1950) showed that in a number of fishes the pineal region has an influence on the behavior of the melanophores and on the reactions of the animals to light and darkness. They broadly distinguished three types of fishes: (1) Those in which the tissues, including the skull, overlying the pineal permit light to enter the cranial cavity freely in this region. Such fish tend to be strongly positively phototactic. (2) Those in which the tissues are so opaque that no light can enter. These fish tend to be strongly negatively phototactic. (3) Those in which the movements of chromatophores control the amount of light which reaches the pineal complex. This last group presents what appears to be an erratic behavior with regard to reactions to light. Rivas (1953, cited in Hoar, 1955) described the pineal apparatus of the tuna (*Thunnus thynnus*) and suggested that it was important in the control of phototactic movements. Hoar (1955) found that the juvenile salmon (*Salmo*) appears to belong to the group of fishes which can cover or uncover the pineal area by movements of chromatophores. The pineal region is clearly sensitive to light and plays a part in the control of the chromatophores and in the typical negative phototaxis.

A particular characteristic of the diencephalon is its relation to the endocrine system. Apart from the obvious external connection with the hypophysis, there are regions of secretion within the diencephalon itself. Scharrer (1928) examined the minnow brain histologically in connection with his experiments on sensitivity to light and, in the course of this work, found apparently secretory tissue. In a series of papers (Scharrer 1930, 1932, 1933, 1952; Scharrer and Scharrer 1949, 1955) he went on to describe neurosecretory cells in the diencephalon and particularly in the nucleus supraopticus and the n. paraventricularis. Further, the work of Bargmann, Held, and Zetler has indicated the presence of special paths which lead secretions produced in the diencephalon to the hypophysis. References to these and other investigations may be found in the papers by Mazzi (1952), Romieu and Stahl (1952), and van de Kamer and Verhagen (1954, 1955).

It was seen in the section on the telencephalon that, in the few species which have been studied, the thalamus fish can scarcely be distinguished from the normal animal. While the telencephalon exerts some control over the rest of the brain, this is by no means obvious without careful observation. In most situations the diencephalon, with other more posterior regions of the central nervous system, is sufficient to decide the activity of the fish without control by the telencephalon. From this fact and from our knowledge of its functions in higher forms we may expect the diencephalon in fishes to be important for the correlation of incoming and outgoing messages, particularly with regard to the endocrine system, the autonomic nervous system, and the general internal relations of the animal. The few experimental results point towards some of these functions but more definite physiological evidence is not yet available.

## IV. THE MESENCEPHALON

### A. Anatomy

The mesencephalon or midbrain of fishes forms a relatively large structure which is divided into the dorsal roof or optic tectum and the ventral tegmentum. The tectum is divided by a longitudinal furrow into two optic lobes (corpora bigemina) which are larger in teleosts than in elasmobranchs (Fig. 1). In teleosts part of the medial region of the tectum extends into the ventricle as paired tori longitudinales. After complete crossing of fibers in the optic chiasma the optic tracts end to a great extent in the tectum. There are also afferent tracts connected with Nerve I (olfactory) from the telencephalon, V (gustatory), VIII (acoustico-lateralis), and the general cutaneous centers of the spinal cord (touch and other cutaneous senses) which enter the midbrain to reach the tegmentum and, in the elasmobranchs, the tectum. In the teleosts a passage of acoustico-lateralis afferent fibers to the tectum does not appear to have been established. The cells in the optic tectum are arranged in layers which form a complicated pattern. Within these layers connections exist between the incoming afferent fibers and efferent tracts which are linked up with the nuclei for the eye muscles and with the motor neurones of the neuromuscular effector system generally. At the same time the midbrain is connected reciprocally with the other brain regions. Thus both tectum and tegmentum are connected with the cerebellum by the tractus tecto-cerebellaris and the tractus mesencephalon-cerebellaris, with the thalamic region and with the medulla and spinal cord by the large tecto-bulbar and tecto-spinal tracts (Kappers, *et al.*, 1936; Tuge, 1934b). All these anatomical facts indicate that the tectum of fishes is concerned not only with vision but also with the correlation of sensory impressions and of body position and movement. Further, comparisons have often been made between the optic tectum and the mammalian cortex, which is structurally somewhat similar, and it has been suggested that the anatomical similarities may be correlated with analogous physiological functions.

### B. The Effects of Lesions and Removal of Parts of the Midbrain upon Posture and Movement

Baudelot (1864) reported that after removal of the roof of the midbrain in the teleost *Gasterosteus* the animal is blind but its movements are not disturbed. His observations were confirmed by Vulpian (1866), Steiner (1888), and Bethe (1899) who found that the balance of elasmobranchs and teleosts is unaffected by unilateral or complete removal of the optic tectum. On the other hand, Ferrier (1876) described irregular movements in fish after superficial lesions of the optic lobes, as did Polimanti (1911, 1912a, b). Reisinger (1915) also found disturbances of balance in the perch (*Perca fluviatilis*) after removing parts of the midbrain while Springer (1928) claimed that section of the optic lobes of some elasmobranchs and teleosts is followed by irregularities in respiratory movements. However, in these last experiments the descriptions of methods are not sufficiently precise to allow comparison with the results of other workers.

Rizzolo (1929b) removed both optic lobes from eight individuals of the elasmobranch *Galeus canis*. In three cases the animals swam normally. In the remaining five cases they swam either to the right or to the left around the dorso-ventral axis, according to the position in which they were placed by the experimenter, and movement became normal within twenty-four hours. Rizzolo therefore concluded that removal of the tectum has no adverse effect upon balance.

More recently Dijkgraaf (1949a) removed the optic tectum, including the tori longitudinales, both on one side only and completely, in the minnow (*Phoxinus*). As long as the operation is carried out without damaging the tori semicirculares, which lie in the upper surface of the tegmentum immediately below the tectum and are connected with it by numerous fiber bundles, Dijkgraaf reports that the animals swim normally, maintain their balance and feed without hesitation. After removal of the optic tectum on one side the fish is blind on the other side and when swimming slowly there is a slight tilting of the median plane towards the seeing eye. A similar tilting can be observed in a minnow with intact brain in which the eye has been removed on one side.

Various experiments have been made involving not only extirpation of the tectum but also of the deeper lying regions of the midbrain. Baudelot (1864) first claimed that unilateral lesions in the base of the midbrain of fishes are followed by rolling movements toward the unoperated side. Vulpian (1866) reported rolling and circus movements in the carp (*Cyprinus carpio*) after similar operations. Traube-Mengarini (1884) used localized lesions and electrical stimulation of the midbrain and concluded that this region contained centers for balance, coordinated movements, and acceleration and inhibition of breathing movements. On the other hand, Steiner (1888, p. 32) did not confirm her results and criticized the methods which had been used, maintaining that the brain, as a result of faulty techniques, had suffered damage through the entry of water. He concluded that the midbrain does not contain coordinating centers but that it receives sensory impressions from proprioceptors and from skin sense organs, leading them to a movement center in the medulla.

Loeb (1891, 1901) found that after removal of one half of the midbrain in *Scyliorhinus canicula* the animal carried out circus and rolling movements with bending of the body towards the undamaged side but that these effects did not appear if Nerve VIII of the same side was cut. Both Loeb and Bethe (1899), who made similar experiments, considered that these abnormal movements were the result of damage to centers associated with Nerve VIII.

Polimanti (1911, 1912a, b, 1913), after unilateral and bilateral lesions in the optic tectum in elasmobranchs and teleosts, found disturbances of balance, circus movements, and abnormal eye movements and pupillary reflexes. When, in addition to the tectum, the deeper lying regions of the midbrain were damaged, the effects were much more pronounced. After complete removal of the midbrain there were great disturbances of movement and balance.

Muskens (1930) made lesions in the midbrain of goldfish (*Carassius auratus*) and noted the forced movements which followed. He distinguished such movements as being predominantly either in the horizontal and frontal planes or in the vertical plane and correlated these types of total movement of the fish with lesions in specific regions of the midbrain. Muskens thus laid stress on the movements of the animal as a whole rather than on those of isolated locomotor organs. In doing so he foreshadowed more recent conceptions of the functions of the midbrain (p. 22).

Rizzo (1929, 1932) concluded from the results of lesions that muscle tone, locomotion, and balance are regulated both from the midbrain and from the cerebellum. Ten Cate (1935) did not consider that Rizzo's methods were wholly satisfactory.

### C. Motor Effects Following Stimulation of the Midbrain

Among the later experiments on the motor significance of the tectum is the work of Chauchard and Chauchard (1927a) who stimulated various parts of the tectum electrically in an attempt to find out whether any relationship existed between a given region and the contraction of specific muscles. In the teleost *Mugil auratus* stimulation of the anterior region of the median surface of one lobe produced movements of the tail towards the opposite side and spreading out of the caudal fin; stimulation of the median surface at the border of the anterior and middle third of the lobe resulted in erection of the hinder dorsal fin; stimulation of the boundary between the middle and posterior third of the lobe was followed by erection of the anterior dorsal fin; laterally and a little behind this point it produced spreading out of the pectoral fin of the other side; on the outer side of the lobe stimulation of the anterior region caused rotation of the pectoral fin of the same side while stimulation of the middle region was followed by movements of the eyes. In *Trigla gurnardus* stimulation of corresponding points on the tectum produced the same movements. This fish has modified pectoral fins, some of the fin rays being separately movable and used for crawling on the bottom. This apparatus was set in action when stimulation was applied to a point on the outer surface of the optic lobe a little in front of the region whose stimulation resulted in movement of the pectoral fin. Similar experiments carried out on the rays *Dasyatis* and *Myliobatis* gave somewhat different results because of the formation of the locomotory apparatus in these animals. Electrical stimulation from the front backward along the middle line of an optic lobe resulted in movement of the tail segments of the opposite side in the opposite direction, i.e. from the free end of the tail towards its base. Stimulation of the anterior end at both sides of this midline produced movements of the anterior region of the pectoral fin; stimulation towards the inside of the midline caused the fin to be raised while stimulation towards the outside of this line caused it to be lowered. These results as well as others of a similar nature were presented by Chauchard and Chauchard as evidence supporting the opinion of comparative anatomists that the optic tectum of fishes has functions which, in higher vertebrates, are associated with the cerebral cortex, both showing a localization of motor areas for specific regions of the body. In a further paper these workers (Chauchard and Chauchard, 1927b) found chronaxie values of the order of 0.0002 second for various zones in the optic lobes of elasmobranchs and teleosts, the values differing slightly among the zones.

Ten Cate (1931c) and ten Cate and ten Cate (1931) have in general confirmed the results of the Chauchards by experiments on elasmobranchs in which curare was applied to small superficial lesions on the surface of the optic tectum: these again indicated a definite functional correlation between the surface of the tectum and prescribed regions of the body. According to ten Cate, damage to portions of the tectum is followed by abnormal carriage of the fins so that the body is inclined to one side and balance is disturbed. Ten Cate has suggested that in these cases there may be a stimulation of the nervous elements which connect the corpora bigemina with the medulla oblongata and that the corpora bigemina of the elasmobranchs appear to play a definite role in the maintenance of balance in these animals.

### D. Relations of the Optic Tectum to Vision and to Motor Activity

Dijkgraaf (1949a), working on the minnow (*Phoxinus*), showed that after removal of the optic tectum of one side and the eye of the same side the animal is completely blind. Some slight reactions which the animal still shows to large objects held above it can be attributed to the shadowing of the unpigmented fleck on the top of the head (Scharrer, 1928; and see p. 14). Wichers continued these experiments in Dijkgraaf's laboratory (unpublished; Dijkgraaf, personal communication). Having blinded ten minnows unilaterally by removing the right eye, she cut out either the whole tectum or different parts of it and studied the resulting defects in the visual field of the left eye. Feeding and fixation reactions were used as tests of vision. Due attention was paid to errors which might arise through chemical and lateral line stimuli. Her results were: (1) the visual field of the left eye in the animal with intact tectum is nearly a hemisphere (Fig. 3); (2) after complete extirpation of the right tectum the animal cannot see with the left eye; (3) after partial extirpation of the right tectum the visual field of the left eye shows defects which correspond topographically with the tectal injury; removal of the anterior part of the right tectum is followed by blindness in the anterior region of the visual field of the left eye; removal of the more posterior tectal region produces blindness in the posterior portion of the left visual field; and similarly for other regions (Fig. 4). Some minor exceptions to this correspondence between tectal surface and visual field result from the fact that the part which is removed inevitably includes nerve tracts running to other tectal regions. Wichers also followed the course of the fibers from the retina to the tectum and obtained similar results.

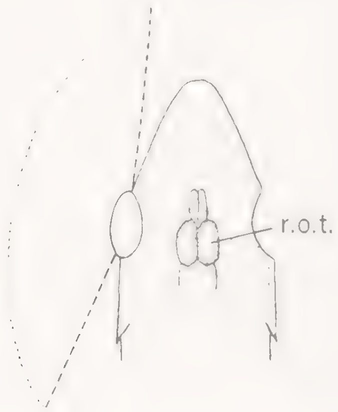

**Fig. 3. Visual field of the left eye of the minnow (*Phoxinus*) seen from above.** r.o.t. = right optic tectum. (Drawn from data supplied by Dijkgraaf, unpublished.)

Although after complete removal of the optic tectum in the normal minnow and unilateral removal of the tectum and of one eye on the same side the fish cannot *see*, it still exhibits color changes mediated through the eye. The paths of the fibers from the retina to the autonomic system must therefore avoid the tectum. Further, Dijkgraaf (1949a) found that the darkening which appears in the normal minnow after removal of both eyes occurs only slightly or not at all if, instead, blinding is accomplished by complete removal of the optic tectum. If in an eyeless, darkened minnow the tectum is removed on one side only, there is no change in the tint of the skin but after removal of the remaining half of the tectum there is immediately a pronounced paling which partially persists. These observations await explanation.

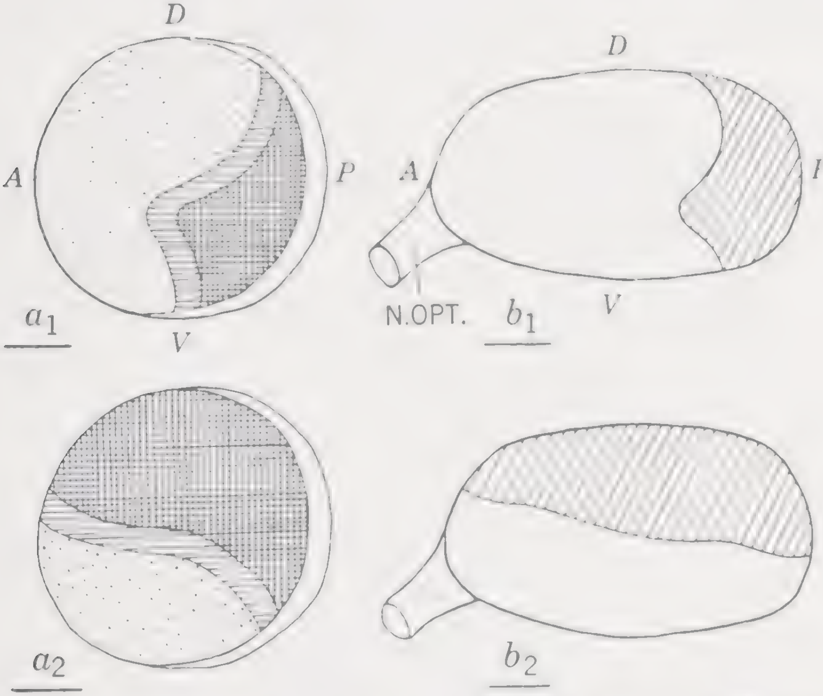

FIG. 4. Defects in the visual field of the left eye of the minnow (*Phoxinus*) after removing various regions of the right optic tectum. A = anterior, P = posterior, D = dorsal, V = ventral. *a:* Visual field of the left eye, seen from the left side. Crossed lines = no vision; single lines = irregular reactions; dots = clear vision. *b:* Mirror image of right optic tectum seen from the side. Lines = region which has been removed. (Drawn from data supplied by Dijkgraaf, unpublished.)

The experiments of Dijkgraaf and Wichers show that a projection of the retina can be mapped upon the optic tectum of the minnow. Such a spatial coordination between the retinal field and the surface of the tectum, or of the colliculi quadrigemini rostrales of higher vertebrates, has been shown both anatomically and physiologically in different animal groups; thus Apter (1945) and Hoessly (1947), working on lower mammals, found relations of this kind.

Hess, *et al.* (1946) showed that electrical stimulation of definite regions of the mesencephalon of the cat could be correlated with typical changes in posture of the eyes and head which were regarded as special "visual grasp reflexes" ("visuelle Greifreflexe") for the perception of objects in motion. Anatomical control by the Marchi method indicated that direct visual projections from the retina to the superior colliculi reach a system of synapses in the tectum. Here an adequate reflex response is organized and mediated by way of the tecto-spinal tract to the motor nuclei connected with eye, neck, and upper trunk. The tegmentum is considered to be concerned with bringing the rest of the body into a stable basic posture and counterbalancing the forces which might impede faultless execution of directed movements brought about by the tectum. Thus there is a definite series of nervous processes associated with looking at an object as a result of which various body movements occur and an object appearing in the periphery of the visual field is involuntarily brought upon the visual axis and clearly seen. On the basis of this work Akert (1947, 1949a,b) investigated functions of the mesencephalon in the rainbow trout (*Salmo gairdnerii*). This fish is very dependent upon its vision and the whole body is concerned in moving to carry out a visual grasp reflex. Akert used a stimulating electrode to explore the dorsal surface of the tectum. In his records Akert differentiated between two voltage ranges of stimulus: 0.8–1.7 v. and 1.8–2.5 v. The recorded place at which the current entered was not necessarily the same as the place where it had its effect, i.e. the latter was not defined merely by the horizontal projection. Apart from the use of these two strengths of stimulus, the localization in depth of the effects could not be more exactly analyzed inside the tectal layers but the liquid in the ventricle certainly prevented stimuli from reaching the tegmentum. Tests on the whole of the dorsal surface of the brain showed that only stimulation of the optic tectum was followed by motor activity. Stimulation of the forebrain and of the cerebellum only produced motor activity when the stimulus was so strong that uncontrollable currents could have been responsible. Typical reactions appeared with stimuli of strength 1–2 v., frequency 15 c.p.s., and duration 2–3 seconds. The effectors of the locomotory apparatus come into action in a definite series which agrees with their relative effectiveness in moving the animal. The excitation passes very quickly in a craniocaudal direction: first eye movements, sometimes snapping of the jaws, then movements of paired and unpaired fins, and finally beats of the tail. The whole succession gives the impression of active movement towards some goal in which both fins and body muscles work together. The eye movements were especially studied and showed that the effect of the stimulus appears after a definite latent period which can last up to 1 second, according to the strength of the stimulus. If the stimuli are slow enough each effect is isolated, but if the frequency is greater than 8–10 c.p.s. the effects of stimulation become added to one another and the eyes take up a fixed position. On stopping the stimulus the eyes maintain their position for a short time and then gradually return to their original position. These summation and after-discharge effects agree with the fact that repeated stimulation with short pauses shortens the latent period and that the stimulating effect of a constant voltage becomes steadily greater. If the stimulation is repeated many times the excitability becomes lowered and finally there is no response. After a long pause the reaction is again obtained. Such phenomena are familiar in neurophysiology. The movements of the eyes can be regarded as pointers to the locomotory apparatus of the total position which must be taken up. Both eyes always move together and, according to their direction, the fins and the muscles of the body take up a position which would bring the fish in that direction (Fig. 5). Figure 6 shows the relation between the direction of

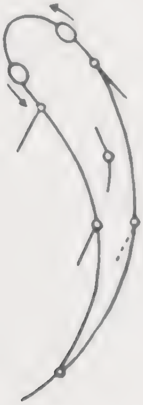

FIGURE 5

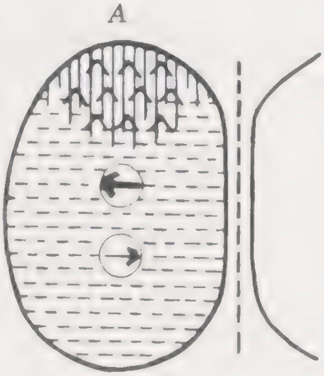

FIGURE 6

FIG. 5. Diagram showing turning to the left induced by the tectum in *Salmo gairdnerii* seen from above. Both eyes are turned to the left, the body is bent so that its concave side is toward the left; corresponding movement of the unpaired and tail fins; paired fins also held so that turning to the left is favored. Stimulus of 1.8 v., 15 c.p.s., applied in the pre-central region of the right tectum (from Akert, 1949a).

FIG. 6. Points on the left optic tectum which are associated with definite eye movements (*Salmo*). Tectum viewed from above. A = anterior; vertical lines = raising. $\wedge$ = convergence; horizontal lines = turning. Thin arrow = turning to right (ipsiversive) (0.8–1.7 v.). Thick arrow = turning to left (contraversive) (1.8–2.5 v.) (from Akert, 1949a).

movement of the eyes and the point of stimulation on the tectum. The region connected with convergence is small but turning of the eyes is associated with all the remainder of the tectal surface. Ipsiversive and contraversive turning are both associated with this whole region, the effect produced depending upon the voltage used for stimulation. The lower voltage range (up to 1.7 v.) produces ipsiversive turning of the eye while the higher range produces contraversive turning. According to Akert the most likely explanation is that the cells responsible for these movements lie at different levels and the higher voltage is necessary to reach those controlling contraversive turning. The figure does not give a quantitative picture, the greatest turning effects being in the central and precentral regions. Raising of the eyes is also associated with a definite region which largely coincides with that associated with convergence. Lowering of the eyes was not observed but, according to Akert, it might possibly be associated with the lower lateral region of the tectum which was not examined because of technical difficulties. Whatever the reactions of the eyes, they were always accompanied by and coordinated with movements of fins and body muscles.

Stimulation of the tegmentum (floor of the ventricle) produced effects essentially different from those associated with the tectum. The threshold of stimulation fell to 0.4–0.5 v., stimuli within this range sufficing to produce an immediate abrupt response of the whole locomotory apparatus without sequence in time and without any gradation. On stopping the stimulation such reactions cease abruptly. There is no impression of coordination or of movement directed to any particular end; the eyes turn to the stimulated side, all the fins expand, and contractions pass in waves over the trunk muscles. These reactions resemble those found in response to similar stimulation of the tegmentum in cats by Hess *et al.* (1946).

These results show that the tectal elements concerned are not connected with single effectors but with a functional system which can control a great variety of muscles, i.e. in the tectum there is not a representation of effectors but of coordinated connections. This opinion is contrary to that of Chauchard and Chauchard (p. 18) who thought that motor functions belonging to specific parts of the body were associated with definite tectal regions. The French workers may have arrived at their conclusions through using a strength of stimulation which caused excitation beyond the immediate tectum and reached actual efferent tracts.

In the case of the trout (*S. gairdnerii*) the movements towards some goal are mainly visually directed, other senses being much less concerned. The eye is thus most important in searching for and snapping at food,

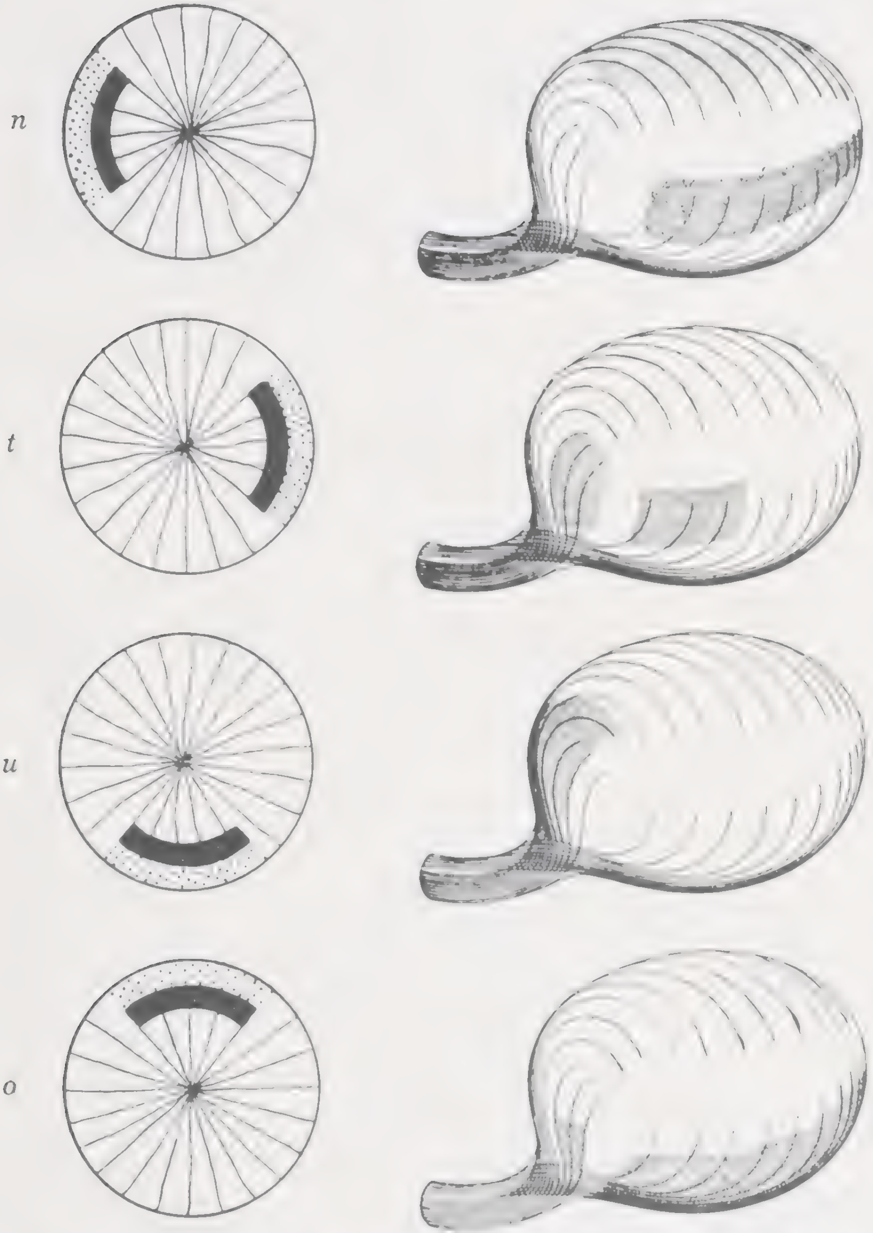

FIG. 7. Diagram showing the projection of the periphery of the visual field of the right eye on the surface of the left optic tectum, as obtained by the Marchi technique. The diagrams on the left show the localization of the retinal lesions. black portions = region of coagulation by diathermy; dotted portions = region of damaged fibers of the optic nerve. The right eye is seen from behind the retina. $n =$ anterior (nasal) lesion; $t =$ posterior (temporal) lesion; $o =$ dorsal lesion; $u =$ ventral lesion. The diagrams on the right show the regions in the left tectum (dotted) where the visual fibers arising from the damaged retinal region have degenerated. The left tectum, drawn as a solid body, is seen from the left side and slightly from the front. The lines represent the visual fibers which enter the tectum (from Akert, 1949a).

especially when feeding on insects at the surface of the water. The functions of the midbrain which have just been described are clearly significant in effecting the necessary coordination of visual perception with body movements. In this connection Akert investigated the central representation of the visual field in the optic tectum. After making retinal lesions he tested the visual field by noting the response to food and then examined the lesions and the nerve fibers passing to the tectum by the Marchi method. Figures 7 and 8 show the degeneration in the tectum corresponding with specific retinal lesions. It can be seen that equally sized peripheral lesions in different parts of the retina do not produce equal areas of corresponding degeneration in the tectum; the areas of degeneration in the tectum are greatest after lesions of the retina in the nasal and temporal regions. However, there is clearly a definite projection of the retina in the optic tectum in S. *gairdnerii*. This projection is field-like and to a certain extent analogous to the grouping of the sensory cells of the retina, although Akert was not able to demonstrate a point to point projection with the methods used. A correspondence between retina and tectum has more than once been shown for fishes which see well whereas

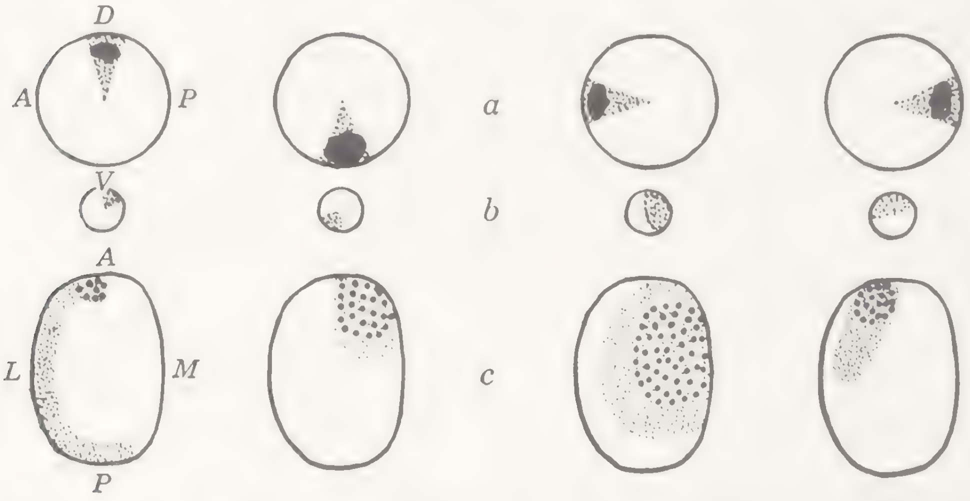

FIG. 8. Diagram showing the projection of the periphery of the retina of the right eye in the optic tract and in the left optic tectum. Top row (*a*) = retinal lesions. The right retina is upright and seen from the back. D = dorsal; V = ventral; A = anterior (nasal); P = posterior (temporal). Middle row (*b*) = relations of the fibers in the optic tract. The latter is shown in section close to the point at which it enters the brain and observed from the side nearer the brain. Bottom row (*c*) = projection in the optic tectum as seen from above. The small dots show the localization of the finely myelinated visual fibers in the second layer; the large dots show the distribution of the more strongly myelinated fibers in the fourth layer of fibers (from Akert, 1949b).

those species whose reactions are not primarily visual, such as *Ameiurus nebulosus,* do not appear to exhibit it (Akert, 1949b). Other investigations of highly visually reacting fish have failed to show the presence of this projection because of the technical difficulties involved (e.g. Meader, 1934).

Such a projection picture can be compared with the results of electrical stimulation of the tectum and Figs. 6 and 9 show that there is a far-reaching coincidence of the optical and motor projection fields. Thus, the projection of the temporal visual field agrees well with that area which is associated with contra- and ipsiversive turning in Akert's stimulation experiments. Akert thus concludes that physiologically this area, excited by a stimulus arising in a corner of the temporal field, brings about a positioning of the eyes and of the body axis so that there is either a turning toward or a turning away from the object perceived.

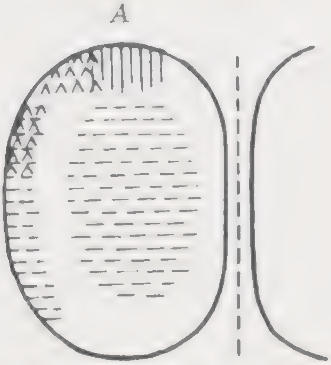

FIG. 9. Diagram showing the projection of the periphery of the visual field of the right eye on the surface of the left optic tectum. Tectum viewed from above. The disposition of all fibers indicated in Fig. 7 is shown, excepting those of the lower visual field. A = anterior; vertical lines = projection of the upper visual field; ∧ = projection of the nasal visual field; horizontal lines = projection of the temporal visual field (from Akert, 1949a).

Akert's work suggests a possible explanation for some of the conflicting observations of earlier workers following removal of parts of the tectum. While some of these observations may have been the result of faulty techniques, it is conceivable that some lesions in the tectum may be followed by positional and motor irregularities rather because they remove certain elements from the total complex modifying system and throw the output of the remainder into a state of unbalance than that they stimulate specific regions. On the other hand, removal of the entire tectum or of one complete lobe may not introduce any unbalanced modification by way of the tecto-spinal tracts.

An interesting hypothesis bearing on the role of the optic tectum in the coordination of vision and movement has been put forward by Sperry (1950a). This is concerned with the forced circling (spontaneous optokinetic) movement which is a conspicuous result of rotation of the eyeball through 180°. Sperry has shown that similar circus movements follow contralateral transplantation of the eyeball with inversion on only one axis and also cross-connection of the optic nerves to the wrong side of the brain. (See Sperry 1950a for references.) As a result of the visual inversion the apparent position and direction of motion of the visual field with relation to the posture and movements of the animal are the reverse of normal. Movements of an animal thus result in a more rapid motion of the visual field in the same direction. The optokinetic response to this movement of the visual field under these conditions is such that the animal turns still more in the same direction. In fishes this circling may continue for days. Sperry (1950a) used these forced circling movements in teleost fish to try to find out something about the central neural apparatus involved in the normal visual perception of space and motion. Using *Sphaeroides spengleri*, he rotated one eyeball through 180° and blinded the other eye, at the same time cutting the extra-ocular muscles of the latter. Then he determined the effects upon the forced circling movements of removal of various parts of the brain (Fig. 10). Since removal of the forebrain, cerebellum, and inferior lobes had no effect upon the circling, the most likely remaining site of the integration center of retinal and kinetic components was in the optic lobes. In fishes with the left eye rotated, right eye blinded, and the brain otherwise intact, excision of both optic lobes or of the right optic lobe alone was effective

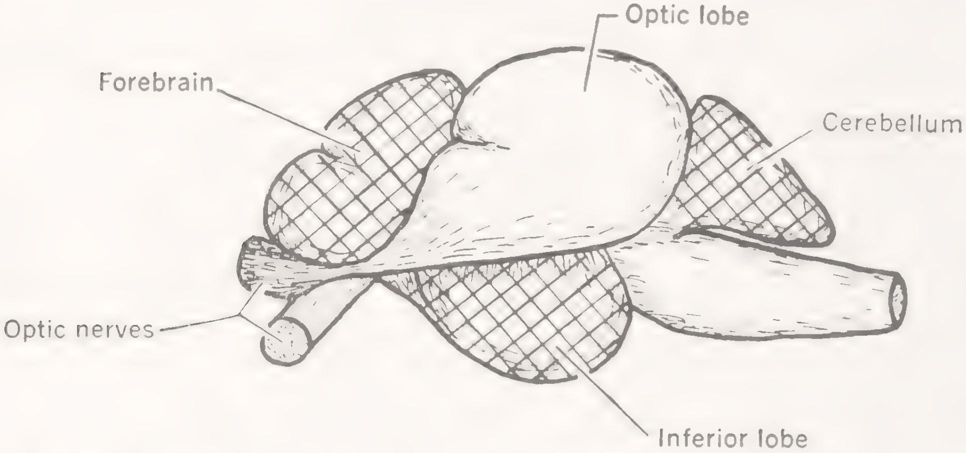

FIG. 10. Diagram of the brain of *Sphaeroides spengleri* (Bloch). Cross-hatched areas indicate the parts removed without disrupting the spontaneous optokinetic reaction (from Sperry, 1950a).

in abolishing the circling response. In three fish with the left eye rotated and the right eye covered with a blinder, removal of the blinder was followed by cessation of the circling. Sperry supposed that the fish responded to the stimuli entering the normal eye while inhibiting the effects of those entering the rotated eye. Excision of the left optic lobe resulted in a resumption of the circling and this continued until the rotated eye was returned to its normal position. The results point to the optic tectum as the controlling center but they "do not exclude the possibility that the optic lobe serves merely as an afferent way-station through which the retinal impulses must pass on their way to an integrating centre at some lower level of the neuraxis. Although not ruled out by the evidence, this seems unlikely in view of the sensory character of the phenomenon" (Sperry, 1950a).

Sperry first assumed that peripheral kinesthetic receptors were responsible for an extra-retinal kinetic component involved in the circling behavior. As the labyrinth seemed the most likely source of such components it was removed on both sides. Following this there was severe disturbance of motor coordination and equilibrium, particularly when the fish tried to swim quickly, but eye rotation still resulted in the characteristic circling. "Combined with the brain lesion data it might be taken to indicate that the circling phenomenon was purely optic and not dependent upon any extra-retinal kinetic factor. An interpretation along these lines could be conceived as follows: With the eye rotated, any movement that is visually initiated or guided, like that involved in centering the gaze on a peripheral object, would only exaggerate, instead of resolving, the stimulating situation which induced it. This in turn would reinforce in cyclic fashion more of the same (erroneous) response. This interpretation has an advantage in that it can be expressed in purely reflex terms without requiring the assumption of an illusory spinning sensation in the animal" (Sperry, 1950a). However, there are difficulties in the way of believing that such regular forced circling movements could be merely a repeatedly frustrated attempt to orientate with respect to some visual object or a succession of such objects. Sperry assumed that there was an illusory spinning of the visual field, such as accompanies visual inversion in man. His conclusions can be best presented in his own words: "Assuming the whirling sensation to be present, it clearly could not result from the retinal stimulation in and of itself. Retinal inversion can exist only with reference to extra-retinal surroundings. It is the inversion with reference to the body and its movement (as registered in the nerve centres) that is crucial. Exactly the same pattern of excitations entering the brain from the retina may, in one instance, arouse the spinning reaction and in another, not, depending entirely upon the direction of the animal's movement accompanying the retinal influx. This is illustrated in Fig. 11. It applies to the whirling sensation in man as well as to the overt spinning of the fish. Thus, the direction of the movement of the perceiving animal is one of the critical factors that condition the central effects of the retinal stimulus."[1]

Since kinesthetic influx from the periphery seems of little importance, in view of the fact that labyrinthectomy caused negligible disturbance although the labyrinths play a dominant role in these animals, Sperry considered another possibility, namely, "that the kinetic component may arise centrally as part of the excitation pattern of the overt movement. Thus, any excitation pattern that normally results in a movement that will cause a displacement of the visual image on the retina may have a corollary discharge into the visual centers to compensate for the retinal

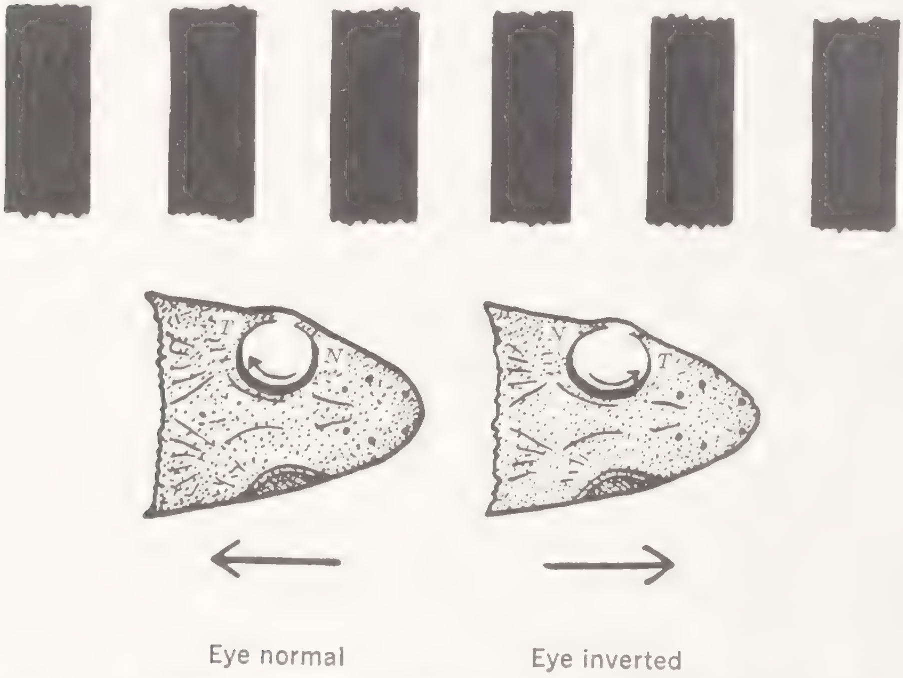

FIG. 11. A normal fish swimming backward is subjected to the same retinal stimulus as a fish with rotated eye moving forward. The same retinal influx that does not cause spinning in the former case, does in the latter because of the direction of movement with which it is combined. N and T mark nasal and temporal poles of the eye (from Sperry, 1950a).

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[1] Thanks are due to Prof. Sperry and to the American Psychological Association for permission to reproduce diagrams and to quote from the *Journal of Comparative and Physiological Psychology*.

displacement. This implies an anticipatory adjustment in the visual centers specific for each movement with regard to its direction and speed. A central adjustor factor of this kind would aid in maintaining stability of the visual field under normal conditions during the onset of sudden eye, head and body movement. With the retinal field rotated 180 degrees, any such anticipatory adjustment would be in diametric disharmony with the retinal output, and would therefore cause accentuation rather than cancellation of the illusory outside movement."

"For the accurate perception of motion and spatial relations it is necessary that the visual centers be kept in constant adjustment with reference to the specific posture of the body and to the speed and direction of all its movements insofar as these affect the visual field. We may imagine the background excitatory state of the visual brain field undergoing continuous fluctuation of an over-all gradient type in response to any movement or shift of posture that affects the direction of gaze. Similar adjustment of the sensorium may be involved as well in the perception of movement and spatial relations in other sense modalities such as audition and cutaneous sensibility. The indication in the present findings that the postural and kinetic adjustment may have a central origin in part need imply no great decrease in the general importance of afferent excitation from kinesthetic and postural receptors in the periphery. These must continue to be regarded as furnishing an important basic background set or frame of reference for all space perception whether it be principally visual, auditory or cutaneous." (For further discussion of forced circling and of opto-motor reactions see von Holst and Mittelstaedt, 1950; von Holst and Schoen, 1954; von Buddenbrock and Moller-Racke, 1952, 1953; Dijkgraaf, 1953a, b.)

In another series of experiments Sperry (1948, 1949) investigated the growth of regenerating optic fibers into the tectum. Using various teleosts, he blinded the animal on one side and rotated the eye of the other side through 180°. He tested the reactions of the fish by observing (a) the optokinetic responses induced by the rotation of a striped drum around the animal in the horizontal plane; (b) the spontaneous optokinetic reactions which follow eye rotation; (c) the ability to avoid obstacles and approaching objects; (d) positive localizing reactions, such as seizing particles and pursuing other fish. Then he cut the nerve of the rotated eye. As in previous experiments on amphibians (Sperry, 1944, 1945), Sperry found that the regeneration of the optic fibers, although haphazard at the point of section of the optic nerve, resulted in the reestablishment of the central associations in the brain in such a way that the relations which had existed before the section of the optic nerve were again present; i.e. the fish still behaved as it did when the eye had first been rotated. (Cutting the optic fibers in an unrotated eye is similarly followed by regeneration of the unrotated condition.) The fact that the formation of synaptic relations took place in this manner, with no regard for functional adaptiveness, indicates that in the teleosts, as in the amphibians, the optic fibers "are qualitatively specified through embryonic differentiation of the retinal field and that this specification determines the types of synaptic associations formed in the centers." (Sperry, 1948). In the time which elapsed before the fish died, some individuals gave indications that they were beginning to adjust their movements but it was not possible to say whether this represented learning or some other form of functional adaptation. (See Sperry, 1950b; Weiss, 1950.)

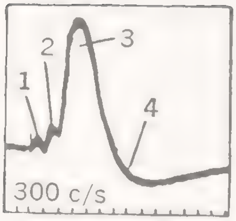

FIGURE 12

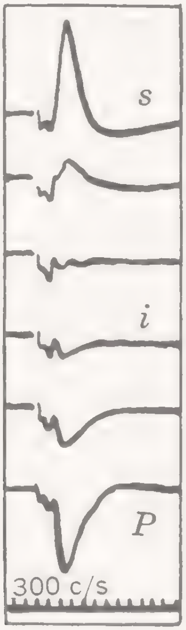

FIGURE 13

FIG. 12. Changes in the electrical potential in the optic tectum of the catfish (*Ameiurus nebulosus*) after electrical stimulation of the contralateral optic nerve. 1,2: fast components; 3,4: negative phase, then positive phase of the slow component. The artifact caused by the electrical stimulation of the optic nerve can be seen to the left of 1 (from Buser, 1955).

FIG. 13. Catfish: Difference in the response of the optic lobe with increasing dorso-ventral penetration of a fine electrode. *s*, trace near the surface of the tectum; *i*, level at which inversion is found; *P*, trace after deep penetration (ventricular surface of tectum) (from Buser, 1955).

## E. Electrical Signs of Activity in the Optic Tectum

In recent years considerable knowledge has been gained of the electrical phenomena associated with tectal activity (Buser, 1949a, b, c;

1950, 1951, 1955; Buser and Dussardier, 1953; Buser and Scherrer, 1950). Working on the catfish, carp, and tench (*Ameiurus nebulosus*, *Cyprinus carpio,* and *Tinca tinca*) Buser showed that an electrical stimulus applied to the optic tract is followed by a complex potential change on the contralateral optic lobe. With an indifferent electrode attached to the body of the animal and a single electrode inserted into the optic tectum the response which is registered consists of two groups of reactions which succeed each other in time. The initial rapid phase of the response consists of two or three brief potentials. These represent the impulses which come along the fibers of the optic tract and spread over the surface of

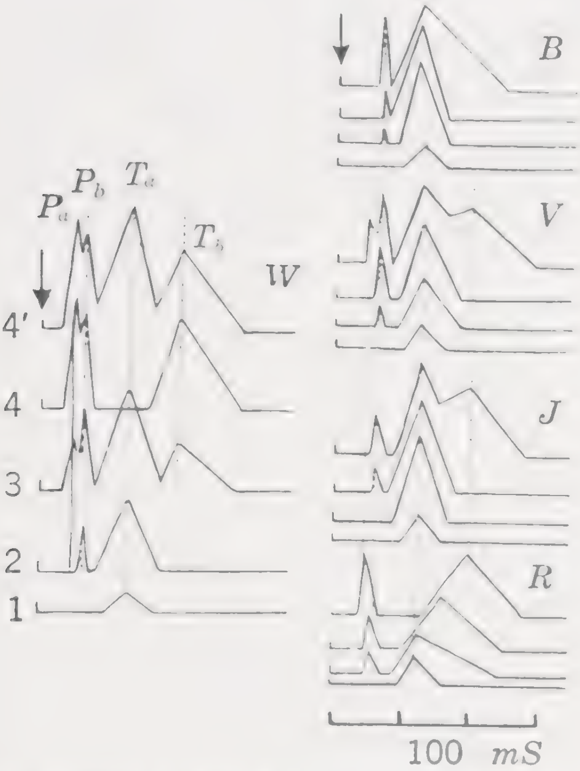

FIG. 14. Catfish: Diagram to show the development of the responses produced in the optic lobe during the progressive increase in intensity of a short light stimulus of known spectral composition. *W*: progressive responses created by stimuli of white light of increasing intensity (1, 2, 3, 4). The arrow indicates the application of the stimulus. 4 and 4′ show the responses which follow identical intense stimuli (more than 30 × liminal) applied in the former under normal conditions and in the latter a short time after extinguishing a continuous light. *B, V, J, R*: the same stages in the responses observed on stimulation with blue, green, yellow, and red light of increasing intensity. *Pa, Pb, Ta, Tb* show the four components which may normally be distinguished in the response of the tectum (from Buser, 1955).

the tectum. Analysis shows that the action potentials of the ramifying optic tract in the tectum are complex in form and exhibit a number of peaks. Once the impulses have passed to the intratectal tangential fibers they are appreciably slowed down so that the rapid phase is followed by one or more slow components. These are diphasic, have a dominant negative initial phase and a greater potential and duration than the first rapid phase (Fig. 12). These post-synaptic components show a complete inversion of polarity between the upper and lower surfaces of the tectum, their principal phase being negative on the upper and positive on the lower surface (i.e. the surface facing into the cavity of the optic lobes). As the electrode is inserted more deeply into the tectum they pass through a minimal amplitude at a level which can be correlated with the histological structure of the tectum for any one slow component in a given species (Fig. 13).

Buser also used the natural stimulus of light on the retina and observed the resulting electrical phenomena in the tectum. He found that a very short intense flash of light covering a large retinal area is followed in the tectum by electrical changes comprising, as before, rapidly occurring components of short duration ("*P*" effects) and one or more slow delayed components ("*T*" effects). In general there are two peaks, $P_a$ and $P_b$, in the early response and two, $T_a$ and $T_b$, in the delayed (Fig. 14). There are indications that the rods and cones of the retina each have associated with them their own *P* and *T* components. When the light stimulus is varied in intensity, duration, or spectral composition the responses in the tectum become progressively modified (Fig. 14).

All these effects essentially characterize the response to a short stimulus. The picture becomes different when we are concerned with the establishment and extinction of a prolonged illumination, although a transition can be seen as the duration becomes less and approaches the time of a brief flash. The changes involved have not yet been defined.

### F. The Relations of the Optic Tectum to Learning and Conditioned Responses

Sanders (1940) trained goldfish (*Carassius auratus*) to respond to a visual stimulus by entering a feeding chamber. He then presented an olfactory stimulus and trained the fish to respond to it by rewarding them with the light signal (although feeding was not allowed). In the goldfish visual learning is mediated through the optic tectum and olfactory learning through the forebrain. Nevertheless, in this case Sanders showed that removal of the optic tectum abolished the response to the olfactory stimulus. The midbrain is thus clearly playing an important part in this second-order learning.

The training of a minnow (*Phoxinus*) to respond to an auditory stimulus was shown not to depend upon the optic tectum by Dijkgraaf (1949a). Complete removal of the tectum on both sides caused no deterioration in the training response to a frequency of 1650 c.p.s.

Sperry and Clark (1949), tried to find out whether habits learned with one eye would transfer without additional training to the other eye. Using a goby, *Bathygobius soporator*, they taught 16 fish a simple visual discrimination with a blinder over one eye. After overtraining, the blinder was shifted to the opposite eye. There was good interocular transfer of the learning in 5 of the 16 cases. Four fishes showed it less well, while the transfer was poor or negligible in the remaining 7. After replacement of the blinder in these last 11 fishes on the untrained eye the learning promptly improved and reached approximately its previous level. In 2 of these the blinder was again shifted to the trained eye and this resulted in a deterioration of the learning in one of them although in the other, in contrast to the earlier result in the same individual, there was excellent transfer. Sperry and Clark concluded that interocular transfer is possible but that the neural mechanisms involved are not sufficiently well developed to allow good transfer automatically in all cases.

Experimental work on the mesencephalon of fishes thus fully supports the conclusions drawn from anatomical studies; namely, that it is a region where incoming messages are received and correlated and then translated into appropriate messages to the effector system. Further, it appears to be concerned with learning. In short, the mesencephalon of fishes exhibits many of the functions which are associated with the cortex of higher vertebrates.

## V. THE METENCEPHALON

### A. Anatomy

The size of the metencephalon or cerebellum in relation to that of the whole brain varies considerably among the fishes. It is very small or practically absent in the cyclostomes but may form a considerable proportion of the bulk of the brain in elasmobranchs, ganoids, and teleosts. In the two latter groups its roof is more thickened than in other forms and projects anteriorly as the valvula cerebelli under the optic tectum. Numerous afferent and efferent tracts enter the cerebellum to link it with the sense organs and with the muscle effector system. Among the afferent tracts may be noted the tracti spino- and olivo-cerebellaris and the tr. mesencephalo-cerebellaris; among the efferent, the tr. cerebello-motorius and the tr. cerebello-tectalis (Tuge, 1934a). The cerebellum, however, contains no primary centers, all its connections with sense organs and effectors being indirect in that paths stretch out from the primary centers to the metencephalon. Thus all reflexes which pass over the cerebellum have a second path independent of it so that the reflex act in every case can also occur without the cerebellum. This fact has probably been responsible for many of the earlier misconceptions of the functions of the metencephalon (Fig. 1).

Two main regions of the cerebellum may be distinguished: the median unpaired corpus cerebelli and the two lateral auricles (auriculi cerebelli). Investigations by Kappers, Wallenberg, and Voorhoeve (see Kappers *et al.*, 1936) have shown that the auricles have a close relation to the vestibular and lateralis apparatus while the corpus cerebelli is particularly connected through the spino- and olivo-cerebellar tracts with the spinal cord, receiving sensory stimuli from the body and communicating with the body musculature.

### B. Early Investigations on the Physiology of the Metencephalon in Fishes

Earlier experiments in which parts or the whole of the cerebellum were removed gave very contradictory results. Renzi (1864) and Dickinson (1865) removed the cerebellum in various teleosts and described great disturbances of balance as a result. On the other hand Baudelot (1864) could detect no change in the swimming movement of the stickleback (*Gasterosteus*) after removing the cerebellum and his results were confirmed by Vulpian (1866) for *Cyprinus* and *Tinca*, by Steiner (1888) for *Squalius cephalus* and *Scyliorhinus* and by Loeb (1891, 1901) for *Scyliorhinus.*

Corso (1895) removed both the entire cerebellum and portions of it from various fishes and found no changes in locomotion or muscular power. Similarly, Bethe (1899), after removing the entire cerebellum or one half of it from the dogfish (*Scyliorhinus*), did not observe any disturbances of movement. However, in other experiments, in addition to removing the cerebellum, he cut through Nerve VIII on one or both sides or cut the medulla oblongata in half behind Nerve X. In these cases he noted a marked tonic contraction of the pectoral fin muscles. He believed that there was some slight contraction when the cerebellum was intact but that it became much more obvious after this region was removed. Polimanti (1911, 1912a, b) found no changes in teleosts after removing the cerebellum but in elasmobranchs he reported some loss of equilibrium and weaker swimming movements after unilateral or complete extirpation.

### C. Physiological Investigations on the Elasmobranch Metencephalon

Tilney (1923) removed the corpus cerebelli in elasmobranchs. He did not observe any disturbance of equilibrium but in some cases the muscular coordination of the body seemed to be greatly impaired and the animals could apparently only move with the aid of their tails. Tilney accordingly concluded that the corpus cerebelli is not concerned with balance but with controlling the synergistic movements of the body musculature, fins, and tail. In later experiments Rizzolo (1929b) also found that removal of the entire cerebellum from *Galeus canis* was not followed by any disturbances in equilibrium.

Ten Cate (1929a,b; 1930a,b; 1931a,b) was the first investigator to obtain consistent results from experiments on the cerebellum in elasmobranchs. He carried out asymmetrical operations on the corpus and auricles separately in a number of species: *Scyliorhinus canicula, S. catulus, Mustelus "vulgaris," Torpedo marmorata, Raja undulata, Dasyatis pastinaca*, and *Myliobatis aquila*. Removal of the auricle on one side only in *S. canicula* was followed by bending of the anterior part of the body toward the operated side. The greater the amount of auricle which had been removed, the further back extended the bending of the body. As a result of this permanent bending the fish made circus movements toward the operated side. If Nerve VIII was cut on both sides before the operation on one auricle, the bending still occurred. It was thus shown to be independent of the labyrinth. Again, section of Nerve VIII resulted in changes in position of the fins and eyes but removal of one auricle was followed principally by an increase in the tone of the body musculature of the same side.

The rays *Dasyatis* and *Myliobatis* swim largely with their pectoral fins. After removal of one auricle the outer edge of the ipsilateral pectoral fin bent upward, i.e. the flexors of the fin became tonically contracted. As a result of this difference in muscle tone the amplitude of the fin beats on the operated side was smaller than on the unoperated side and the animal in consequence circled toward the operated side when swimming. Thus in both sharks and rays the extirpation of one auricle resulted in an increase in tone of the musculature of the same side.

Ten Cate also removed the corpus cerebelli in part or in its entirety. No obvious changes followed extirpation of pieces from the anterior, lateral or posterior regions, of the entire anterior or posterior or right or left halves or of the whole of the corpus cerebelli. Thus, in this respect, his results agreed with those of Steiner, Loeb, Bethe, and Rizzolo. When, however, the regions which were removed included the cerebellar peduncle, changes in the musculature were observed which were very similar to those following extirpation of the auricles. Thus, after taking away the corpus cerebelli and the peduncle, the head was turned tonically upward while if the peduncle was removed on one side only there were ipsilateral increases in tone in the body and fin musculature with consequent circus movements toward the operated side.

In agreement with these results no visible response followed electrical stimulation of the corpus cerebelli in rays. If, however, the side of the peduncle was stimulated there were slight movements of the ipsilateral pectoral fin and sometimes also of the contralateral fin although true tonic contractions were not obtained. Again, while curare had no obvious effect when applied to the corpus cerebelli, its application to the peduncle in *Scyliorhinus* was followed by tonic contractions in the ipsilateral trunk musculature so that the body became bent toward the curarized side. Cocaine had the opposite effect and ten Cate assumed that this substance paralyses the regions which are stimulated by curare.

In further experiments with *Scyliorhinus catulus* ten Cate localized the regions of the peduncle which were affected by lesions. If the lesion was in the hinder part of the peduncle, the symptoms were more or less the same as those which followed damage to the whole organ. Lesions in the anterior surface of the peduncle had much less effect. Ten Cate suggested two possible anatomical explanations: firstly, that the more posterior lesions might well damage the nucleus lateralis cerebelli which lies in this region of the peduncle; secondly, that most of the fibre tracts which connect the corpus cerebelli with the medulla oblongata and the spinal cord run in the hinder part of the peduncle and would be interrupted by destruction of this region. Since the effects of lesions in the hinder peduncular region were similar to those following damage to an auricle, ten Cate suggested that in both cases there might have been damage to the tr. cerebello-motorius cruciatus and rectus which arise both in the auricle and in the corpus cerebelli (Kappers *et al.*, 1936).

Ten Cate's experiments did not explain the particular function of the corpus cerebelli but he concluded that the cerebellum as a whole in fishes is concerned not so much with the maintenance of balance as with the maintenance of a tonic influence on the voluntary body musculature. At the same time, however, it can affect the maintenance of balance through this tonic effect on the musculature.

### D. Physiological Investigations on the Teleost Metencephalon

Reisinger (1915, 1919, 1925, 1926) described disturbances of equilibrium in the perch (*Perca fluviatilis*) after he had removed the entire cerebellum or had made cuts as nearly as possible through some of the major tracts coming to and from the cerebellum. In other experiments he removed the mesencephalon and attributed the very great disturbances of balance which followed this operation to injury to the valvula cerebelli. Reisinger described his perch after the operation as swaying to the right and left, particularly when they were swimming quickly. The eyes were turned downward and the animals appeared to be in a state of continuous unrest. Reisinger himself realized that, apart from the experiment in which there was deliberate injury to the mesencephalon, these disturbances of equilibrium might have resulted from unavoidable damage to structures other than the metencephalon.

Tuge (1934a) studied the effect of removal of the corpus cerebelli in the goldfish (*Carassius auratus*). After the operation there was lack of muscular tone, the movements of the animal were disordered and violent reactions followed mechanical stimulation. Similar disordered movements followed partial extirpation of the corpus cerebelli or lesions in its dorsal surface. Tuge's observations support those of Reisinger and suggest that the cerebellar mechanism in teleosts may be different from that in elasmobranchs. On the other hand, Dijkgraaf (1949a) removed the corpus cerebelli from minnows (*Phoxinus*) and failed to note any disturbance of balance although his fish did resemble Tuge's goldfish in the occasional violence of their behavior. Most of the time they stayed motionless in a corner but suddenly they would rush about the aquarium as if they were very frightened and would bump heavily against the walls without any apparent external stimulation. The significance of this violent behavior is not known.

Karamian (1949) removed the corpus cerebelli from four different teleosts: *Carassius*, *Cyprinus*, *Perca*, and *Esox*. He, too, found disturbance of both equilibrium and locomotion and, in addition, of sensory functions. These effects of the operation were strongest in *Perca* and *Esox*, weak in *Carassius*, and absent in *Cyprinus*. Karamian further found that after removal of only half of the corpus cerebelli in *Carassius* equilibrium was disturbed, the body became crooked and there were, in consequence, circus movements. These various disturbances disappeared within about a month. Removal of only the valvula cerebelli in *Carassius* was followed by locomotory disturbances together with considerably impaired vision and hearing. After removal of both corpus and valvula cerebelli there was great disturbance of balance and locomotion as well as impairment of the senses of touch, pain, vision, and hearing. Hardly any improvement was observed in the course of a month.

These experiments by Karamian are significant in two respects: firstly, the different results following removal of the entire corpus cerebelli in *Perca* and *Cyprinus* agree with those of Reisinger (1915, 1919, 1925, 1926) and Vulpian (1866) for these two fish, respectively; the fact that a disparity between different species has been found by the one investigator suggests, once again, that generalizations may be unwarranted. Secondly, the reported disturbance of sensory functions following operations on the cerebellum opens interesting lines of enquiry.

With reference to electrical activity, Hoagland (1933a,b,c,d; 1934) recorded the spontaneous discharge of nerve impulses from the lateral lines of various fishes and, in *Carassius* and *Perca*, followed these impulses into the cerebellum. Apart from these experiments little appears to have been attempted in this direction.

### E. Anatomical Indications of the Possible Function of the Corpus Cerebelli

Anatomical studies have provided some possible clues to the function of the corpus cerebelli. Thus Edinger (1908), Franz (1911, 1912a,b, 1920), and Tilney (1923) concluded that there is a parallel between the degree of its development and the locomotor activity of the fish. According to them, in those fish which swim poorly the corpus cerebelli is only slightly developed (e.g. *Hippocampus*, *Lophius*, Pleuronectidae generally and, as an extreme case, *Myxine*), while in those which are good swimmers it is well developed (e.g. *Thunnus*, *Scomber*). Thus it was thought that the corpus cerebelli is concerned with the precise control of the voluntary muscles, both in the optimal position and in the carrying out of movements, so that the carriage of the animal is continually being modified and maintained. According to Voorhoeve (1917, cited in Kappers, *et al.*, 1936) this relation between the size of the corpus cerebelli and the activity of the animal is complicated by the fact that there is also a relation between the absolute size of the body and the relative size of the corpus cerebelli.

The Mormyridae tend to be sluggish fish but the cerebellum is of considerable size. This is chiefly because of the greatly developed valvula cerebelli which, according to Herrick (1905), contains a tract conveying chemical impressions from Nerve VII to the cerebellum. Further, since these fish produce weak electrical pulses of characteristic frequencies and are aware of pulses from individuals of the same species, it has been suggested that their large cerebellum and their large Nerve VII may be related to the perception of these electrical signals. Franz (1912b) believed that the cerebellum of fishes might be a center for associating impressions from sensory regions and transmitting appropriate messages along efferent paths and that there might be a far-reaching functional analogy between it and the cerebral cortex of higher vertebrate forms. In further support of this belief he referred to another afferent tract, the tr. mesencephalo-cerebellaris, which enters the cerebellum and is probably connected with vision.

### F. Physiological Investigations on the Role of the Metencephalon in the Formation of Associations

Franz's suggestion that the cerebellum in fishes might have functions analogous to those of the cerebral cortex of higher vertebrates did not receive support from the work of Nolte (1933). This investigator found that even considerable cerebellar lesions in the minnow (*Phoxinus*) had no effect on previously formed color associations and concluded that the strongly developed cerebellum in fishes does not serve as a center for higher associative faculties. Dijkgraaf (1949a) similarly found that after removal of the dorsal part of the corpus cerebelli in the minnow it is still possible to train the animal to form associations with sound and color stimuli. On the other hand, Karamian (1949) reported that after removal of the corpus cerebelli in *Carassius* conditioned reflexes to light were greatly impaired and that after removal of the entire cerebellum it was not possible to form such conditioned reflexes at all. These experiments were made by Karamian in an attempt to trace the evolution of the functional reciprocity which exists between the cerebellum and the cerebrum of mammals. He found that removal of the telencephalon had no effect upon these conditioned reflexes (see p. 7) and that the removal of the cerebellum in fish without the telencephalon or of the telencephalon in fish without the cerebellum gave no indication of any functional relationship between these two regions of the brain in these animals.

The small amount of experimental work which has been done on the physiology of the metencephalon in fishes indicates a role concerned with the maintenance of posture. The idea that it may play any part in the integration of sensory and motor functions and the formation of associations is hardly more than speculative in view of the slight evidence available and its contradictory nature. At the same time, the fact that the anatomy and histological structure of the metencephalon are very similar in all the vertebrates suggests that some of the functions now known to be associated with the cerebellum in higher forms may also be associated with the cerebellum of fishes. Thus, we now know that in mammals the motor, tactile, auditory, and visual areas of the cerebrum project onto related areas in the cerebellum and that these, in turn, project back onto the cerebrum; that stimulation of certain areas of the cerebellum can result in localized movements and suppression or facilitation of cortically or reflexly induced movements; that the cerebellum can influence the sensory and motor centers in the cerebrum, diencephalon, mesencephalon, and medulla oblongata, most probably by raising or lowering the threshold of their excitability; in short, that the cerebellum may be regarded as "the great modulator of neurologic function" (Snider, 1950). Although this cannot yet be said of the cerebellum of fishes there are indications that this or a similar conception may in time be found to apply.

# VI. THE MEDULLA OBLONGATA AND SPINAL CORD

## A. The Medulla Oblongata

### 1. Anatomical Relations of the Medulla Oblongata

The medulla oblongata is much more obviously derived from the spinal cord than are the other parts of the brain. Posteriorly it gradually merges into the spinal cord without any very clear boundaries. Like the spinal cord it has both dorsal and ventral roots, the former very well developed, the latter poorly, forming the cranial nerves V–X.

Nerve V, the trigeminus, has three branches. The ophthalmic and the maxillary are purely sensory and supply, respectively, the dorsal region of the head and the region of the upper jaw. The third main branch, the mandibular, is mixed, sensory fibers supplying the teeth and the skin of the lower jaw and motor fibers innervating the muscles of the lower jaw.

Nerve VI, the abducens, is purely motor and supplies the external recti muscles of the eyes.

Nerve VII, the facialis, has five main branches. The ophthalmic, buccal, and external mandibular branches supply sensory fibers to the lateralis system and taste buds of the snout region and lower jaw. The palatine branch innervates taste buds in the roof of the mouth. The mixed hyomandibular branch innervates the muscles and skin of the spiracular and hyoid regions. In those fishes in which the taste buds extend over the back and flanks of the animal there is an associated hypertrophy of the visceral sensory roots of the facial nerve (see Kappers *et al.*, 1936, p. 354; Scharrer *et al.*, 1947; Torrey, 1934).

Nerve VIII, the acusticus, supplies the ear and the labyrinth with sensory fibers.

Nerve IX, the glossopharyngeus, is mixed, sending motor fibers to the muscles of the first branchial arch, sensory (gustatory) fibers to the mouth cavity, and some sensory fibers for touch to the head.

Nerve X, the vagus, contains sensory and motor fibers going to a variety of regions of the body: the lateral line, taste buds in the mouth, touch on the skin, and visceral sense organs; branchial muscles, heart, stomach, and intestine. These various fiber systems are all referred to as parts of Nerve X but, once within the medulla, they become separated and are connected with their individual points of origin.

The medulla oblongata is thus associated with both sensory and motor fibers serving widely different parts of the body. While in the higher vertebrates there are developed long fiber tracts leading directly from supra-medullary brain centers to the spinal cord, in the fishes the medulla oblongata appears to be largely a relaying station between the spinal cord and these centers. Apart from these differences the medulla oblongata appears to be very uniform in general structure and function throughout the vertebrate series.

## 2. Physiological Investigations on the Medulla Oblongata

*a. Equilibrium and the acoustico-lateralis system.* Early experiments by Flourens (1842) and Baudelot (1864) on the physiology of the medulla oblongata in fishes confirmed its importance in connection with the maintenance of equilibrium. After making cuts in the medulla of teleosts Baudelot observed circus and rolling movements toward the operated side when the animal was swimming and a steady inclination toward the operated side when it was at rest. These observations were confirmed by Vulpian (1866) and Steiner (1888). Loeb (1891), working on the dogfish (*Scyliorhinus*), obtained similar results. He observed that when a cut was made on the right side at the point of entry of Nerve VIII, the eyes turned toward the right and the pectoral fins toward the left. If the cut lay further forward, there were chiefly circling movements and bending of the body toward the operated side. If, on the other hand, the cut was made behind the point of entry of Nerve VIII, rolling movements predominated. Loeb ascribed his results to damage to the medullary connections of Nerve VIII.

Bethe (1899) confirmed Loeb's work. He found that unilateral section of the medulla oblongata at its hinder end was followed by weaker tone of the muscles on the operated side so that the animal turned in circles away from this side. He also observed the effects of double cuts at different levels in the medulla and concluded that the descending vestibular tracts on both sides become mixed in the middle parts of the medulla and that both crossed and uncrossed vestibular fibers are present on both sides of the spinal cord.

Variations of electrical potential in the medulla oblongata, arising from the activity of the lateralis system, were investigated by Hoagland (1933a,b,c,d; 1934) in the catfish (*Ameiurus*), trout (*Salmo*), goldfish (*Carassius auratus*), and perch (*Perca fluviatilis*). He registered continuous discharges from the lateral line sense organs and was able to record them also in the medulla oblongata (see Denny, 1938). Woldring and Dirken (1951), working on the carp (*Cyprinus carpio*), recorded volleys of spike potentials in the acoustic tubercles following slight sounds made near the fish.

*b. Respiratory movements.* Flourens (1842) cut the medulla oblongata on one side in the carp (*Cyprinus*), the pike (*Esox*), and other teleosts and found that the operculum of the same side stopped moving. If he made a cut in the middle of the medulla the movements of both opercula ceased. Similar observations were made by Baudelot (1864), Vulpian (1866), and Steiner (1888).

Hyde (1904) investigated the medullary respiratory center in the skate (*Raja*). A lesion in the medulla was followed by a reduction or even by cessation of some or all of the respiratory movements for about fifteen minutes. After this period movements began once more in stages, one gill or spiracle starting alone and the others coming into action gradually. If the medulla oblongata was separated both from the spinal cord and from the regions of the brain anterior to it there was a pause after which normal respiratory movements were resumed. The coordinated rhythmical activity of the respiratory center was thus shown to be completely independent of the regions of the central nervous system anterior and posterior to the medulla oblongata. Further, if the medulla was divided into right and left halves by a median cut, each half continued to effect coordinated respiratory movements, although these could differ in rhythm on the two sides and sometimes appeared first on one side and only later on the other. Hyde was able to demonstrate that the respiratory movements in the skate are segmental processes and that the respiratory center occupies definite sensory and motor regions of the medulla. The sensory cells, comprising neurones of Nerves VII, IX, and X, are situated in the lobus vagi while the motor cells and their fibers lie ventrally in the medulla. Each ganglion controls the activity of the respiratory muscles to which it is segmentally related but, at the same time, it can initiate impulses which produce coordinated rhythmical respiratory movements. By means of suitable cuts these ganglia and their associated respiratory mechanisms can be separated into two or three groups. Sometimes these continue their activities in phase after their separation. At other times they develop independent rhythms. Thus the spiracle and the first gill arch still exhibit clear respiratory movements when their central organs (nuclei of Nerves VII and IX) are isolated by cuts from the remainder of the central nervous system. Similarly the last four gill arches can carry out respiratory movements when they are isolated with their central apparatus (Nerve X).

Kouliabko (1906) was the first to attempt to determine the conditions which are essential for the normal functioning of the respiratory centers in the medulla oblongata of fishes. By passing a stream of oxygenated Locke-Ringer solution through the isolated head and recording the opercular movements, he showed that the respiratory center in lampreys, ganoids, and teleosts could remain effective for hours. If the supply of Ringer solution was stopped the respiratory movements soon ceased. Kouliabko concluded that the nerve centers are sensitive to lack of oxygen and to accumulation of carbon dioxide and other metabolic byproducts in the blood. Lutz (1930a,b,c), working on the respiratory center of the dogfish (*Scyliorhinus canicula*), found that it could remain active for $3\frac{1}{2}$ hours after stopping the flow of water over the gills and for more than 1 hour after stopping the flow of blood through the aorta. He therefore believed the center to be autonomous and to be to a fair extent independent of normal chemical stimuli in the blood.

Arnaudet *et al.* (1929), Binet and Cardot (1929), and Binet *et al.* (1931) isolated the head of *Gobius lota* and perfused it with different solutions, finding a Ringer which kept the center active for 30 minutes. They then determined the effects on the respiratory center of hydrogen ion concentration and found that activity was best around neutrality. Further, they found that after the opercular movements had been stopped by chloroform they could be started again if caffeine was added to the perfusing fluid.

Lutz, in other experiments (1930d), showed that the respiratory center in *Scyliorhinus* is associated with the cardiac branch of the vagus nerve in that various peripheral stimuli which cause reflex inhibition of the heart in many cases also inhibit the respiratory center. This combined cardiac and respiratory inhibition can still be produced 1 hour after section of the aorta and must therefore be the result of some purely nervous process brought into action by the peripheral stimulation.

The classical experiment (Klopfversuch) in which Goltz showed that tapping on the stomach of the frog could cause reflex inhibition of the heart was repeated with fishes by Bonnet and Abel (1931). They found that removal of the brain in front of the medulla oblongata and of the cerebellum had no effect upon this reflex and concluded that the afferent path runs in the gastric branch of the vagus, the efferent in the cardiac branch.

Changes of electrical potential associated with the activity of the medullary respiratory center in fishes were first recorded by Adrian and Buytendijk (1931), using the isolated brain of the goldfish (*Carassius auratus*) with the forebrain removed. Amplified recordings on an oscilloscope showed periodic potential variations lasting about $\frac{1}{4}$ second and appearing at intervals of 1–3 seconds, the frequency of these variations agreeing with that of the respiratory movements of the fish. Since the isolated preparation was not receiving afferent stimuli it was concluded that these potential changes were the result of spontaneous automatic excitations within the respiratory center itself. Moreover, the slow monophasic course of these waves indicated their generation not through the summation of rapid separate action currents but rather through slowly occurring intracellular processes. (Smaller action potentials of higher frequency were referred by Adrian and Buytendijk to activity of the midbrain.) Woldring and Dirken (1951) explored the medulla of the anesthetized carp (*Cyprinus carpio*) by inserting bipolar microelectrodes. They found considerable electrical activity in the central part of the medulla oblongata but very little in large parts of the vagal and facial lobes. Volleys of spike potentials in respiratory rhythm were detected in two small strips near the midline at the frontal border of the facial lobe just behind the acoustic tubercles. These volleys were associated with inward movement of the gills. Histological examination indicated that the probable source of these discharges was the neurones of the cranial motor nerves VII, IX, and X.

*c. The control of chromatophores.* Von Frisch (1911a) traced the path of the fibers which are responsible for active aggregation of the pigment in the melanophores of the minnow (*Phoxinus*). By studying the chromatic reactions of the fish after section of the spinal cord and medulla at different levels combined with electrical stimulation he was able to show that these fibers arise from the medulla. He concluded that there is a "paling center" (Aufhellungszentrum) in this region which is responsible for active melanophore aggregation (see Chap. VIII). (For further references to the medulla oblongata, see p. 13.)

## B. The Spinal Cord

### 1. Anatomical Relations of the Spinal Cord

In the vertebrate spinal cord there may be grossly distinguished firstly those fibers which remain within the cord itself and are concerned with the spinal reflexes and, secondly, those which connect the cord with various regions of the brain—the ascending and descending fibers. In passing up the vertebrate series from the fishes to the mammals the number and complexity of connections of these fibers increase progressively and, together with this, the dependence of the cord upon the brain becomes ever greater. In the fishes the simpler condition is found. Ascending fibers from the spinal cord only reach the medulla oblongata and the mesencephalon while fibers descending to the cord are only received from the nuclei vestibularis and lateralis in the medulla oblongata and, to a small extent, from the metencephalon. Descending fibers from the telencephalon, diencephalon, and mesencephalon first pass to coordinating and relaying centers in the medulla oblongata before their impulses can affect the spinal cord itself.

### 2. Segmental Innervation of the Skin, Muscles, and Chromatophores

Segmental innervation formed the subject of a number of earlier investigations and has been well treated in the review by ten Cate (1935).

Van Rijnberk (1904–1905) used Sherrington's method of determining the "remaining sensibility" in order to investigate the segmental innervation of the skin in the dogfish *Scyliorhinus catulus*. He severed three consecutive pairs of dorsal roots anterior to a single dorsal root which was allowed to remain intact. Posterior to this intact root he severed three more consecutive pairs of dorsal roots. The area of skin which retained sensibility after this operation represented the full extent of the sensory dermatome of the intact root. Fig. 15 indicates that this area forms a trapezium with unequal parallel sides. Van Rijnberk found that the sensitivity to stimuli is greater in the ventral region of the dermatome than in the dorsal region. He also found that after cutting two dorsal roots a desensitized skin region is formed passing as a band from the dorsal midline, slanting slightly posteriorly, and not reaching the ventral midline. If only one dorsal root is severed no insensible region of the skin is produced at all. This indicates an overlap in the dermatomes which is more strongly emphasized ventrally because of their greater breadth in this region. Van Rijnberk (1904–1905, 1917) similarly demonstrated the presence of an overlapping cranial-caudal arrangement of dermatomes in the pectoral fins of *S. catulus*.

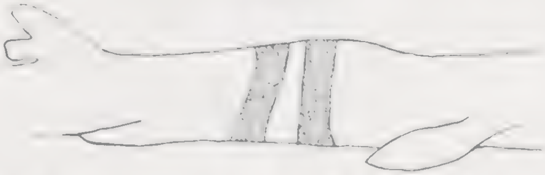

FIG. 15. An isolated dermatome in the dogfish, *Scyliorhinus catulus*, between two analgetic zones (the latter shaded) (from van Rijnberk, 1904–1905).

Ten Cate (1927, 1928a) used the same method to determine the segmental innervation of the skin in another elasmobranch, the ray, *Raja clavata*. As in *S. catulus*, each dermatome forms a trapezium which is wider ventrally than dorsally. Fig. 16 shows the way in which the dermatomes are arranged with an overlap on the dorsal surface.

Among the teleosts similar investigations have been made by van Rijnberk (1905b,c) on *Solea* and *Rhomboidichthys* (= *Bothus*) and by van Herk (1929) on *Platichthys*. The sensory dermatomes in these fish are band-shaped and segmentally arranged, following one another without interruption by the development of the fins. The 12th or 13th dermatome stretches right over the body from the dorsal to the abdominal fin. The more cranial and caudal dermatomes become progressively more V-shaped, the point of the V being directed toward the 12th or 13th dermatome.

Few physiological investigations have been made on the segmental innervation of the effectors of fishes. Müller (1909) applied very weak electrical stimulation to the ventral roots of the elasmobranch *Squalus* and noted only the clearest muscular contractions, ignoring any others. He succeeded in making two, three, or four myomeres contract but never one alone. In the ventral regions the numbers which contracted were even greater. Ten Cate (1935) obtained the same result when he stimulated the ventral roots of *Scyliorhinus*. In the rays he found (ten Cate, 1927, 1928a) that the muscles of the pectoral fin are spread out in one plane and that the motor nerves have the same kind of distribution as that of the sensory nerves described above. Braus (1909, 1910a,b; 1911) found that in *Raja* and *Torpedo* the fin muscles are innervated in order by the spinal nerves with a clear overlap of the motor fields. According to Braus one spinal nerve innervates from six to eight muscles of the pectoral fin, a conclusion questioned by Müller (1909, 1911) and Goodrich (1906, 1910).

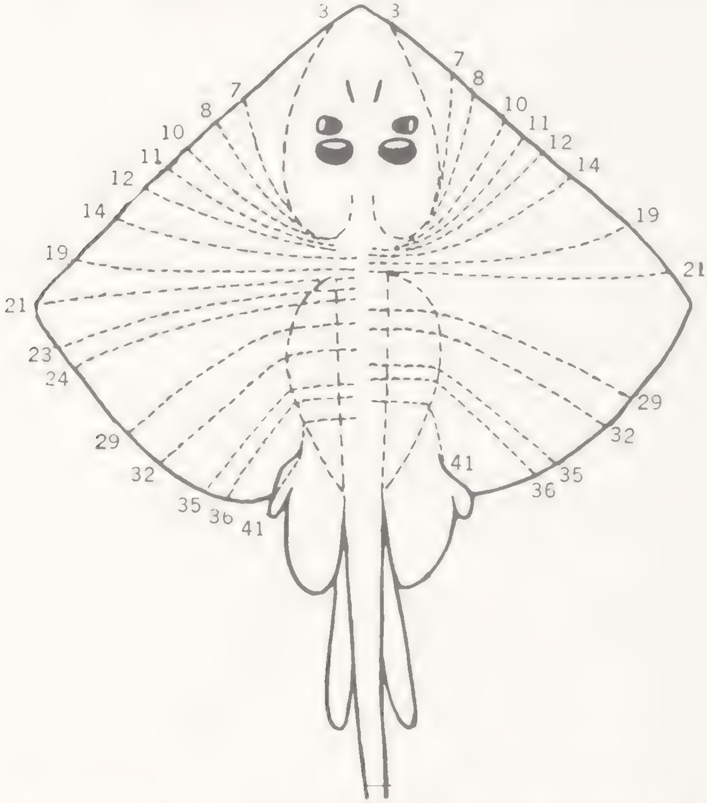

FIG. 16. Boundaries of the dermatomes on the dorsal surface of *Raja clavata*. On the right are shown the cranial boundaries and on the left the caudal boundaries (from ten Cate, 1927).

The segmental innervation of the chromatophores in teleosts has received more attention because it is much easier to investigate. In teleosts the fibers which control active aggregation of the pigment in innervated chromatophores pass from the sympathetic chain into the spinal nerves and so reach the skin. If a few spinal nerves are cut, the resulting interruption of the path of the chromatic fibers is followed within a few seconds by the dispersion of the chromatophore pigment so that a readily discernible dark or colored area appears on the skin of the fish (p. 98). Van Rijnberk (1906b, 1907) used this reaction of the chromatophores together with Sherrington's method in order to investigate their segmental innervation. He found that the "pigmento-motor" dermatomes (van Rijnberk) agreed with the sensory dermatomes in their arrangement, form, and extent and that they also showed an overlap. Van Herk (1929) confirmed these results in *Pleuronectes flesus* (= *Platichthys flesus*).

### 3. Aspects of Excitability and Conduction in the Spinal Cord

*a. The excitability of the spinal cord.* Rizzolo determined the chronaxie at different positions on the spinal cords of two dogfishes. In *Scyliorhinus canicula* (Rizzolo, 1927b) he found a mean value of 0.36 which did not vary in different regions. In *Galeus canis* (Rizzolo, 1930) he chose definite points in the neighborhood of the fins where excitability was greatest, as indicated by fin movements, and again concluded that the chronaxies of these optimal motor points were the same on the dorsal and ventral surfaces of the spinal cord. Further, the chronaxie did not alter with distance from the brain and was unchanged for 6–12 hours after spinal section at the level of the first gill cleft. Rizzolo therefore concluded that the higher centers of the brain do not exercise any steady inhibition or excitation upon the spinal centers. He ascribed the reduction in excitability after longer periods to trauma, disturbance of the blood supply, and entry of sea water into the tissues of his operated animals.

Chronaxies of the same order were recorded by Cardot and Varé (1928) for the spinal cords of various teleosts.

*b. The effects of some poisons upon the spinal cord.* Rizzolo (1927a,c) applied various poisons to the dorsal and ventral surfaces of the spinal cords of several fishes. In the sting-ray *Dasyatis pastinaca* he showed that the chronaxie is not altered when strychnine is applied to the ventral surface but that it is reduced by 50–80% when the drug is applied to the dorsal surface. [For further discussion of the possible significance of this result see Umrath and Hellauer (1948) and Florey (1951).] In further experiments Rizzolo again found that the dorsal and ventral surfaces reacted differently. Thus 1% solutions of cocaine, morphine, and nicotine had no effect upon the chronaxie when they were applied to the ventral surface of the cord but when they were applied to the dorsal surface the chronaxie first became less and then, after some time, greater than normal. Later (Rizzolo, 1929a), he reported that caffeine and theine had no effect upon the chronaxie when applied to the dorsal surface of the spinal cord of these same fishes but that they decreased it when they were applied to the ventral surface.

Blume (1930) found that application of strychnine to the spinal cord of the teleost *Carassius carassius* was followed by a general raising of reflex activity which persisted after removal of parts of the brain and after spinal section. He did not observe any symptoms of cramp. He also injected phenol into a number of teleosts and produced heightened reflexes and cramp symptoms which were unaffected by spinal section.

Bachrach and Binet (1928) poisoned various teleosts with picrotoxin and decided that it attacks the spinal cord, causing selective reduction of certain reflex activities followed later by respiratory and cardiac paralysis.

*c. The spinal cord as a conducting organ.* The various conducting paths of the spinal cord in fishes have been relatively well studied anatomically. The most important tracts are described by Kappers *et al.* (1936). Physiologically, however, conduction within the spinal cord of fishes has been only little investigated. The slight knowledge which we have in this field is confined almost entirely to Mauthner's cells, the passage of impulses controlling the discharge of electric organs and the chromatic effector system. Any account of the nervous control of chromatophores must be concerned with the autonomic system and the conducting role of the spinal cord in this connection can best be described later (p. 97).

*i. Mauthner cells.* The Mauthner cells are two large neurones whose cell bodies lie in the medulla at the level of the root of Nerve VIII and whose axons reach the tip of the spinal cord in the tail. Evidence from Amphibia suggests that the Mauthner cells play a part in coordinating swimming movements along the body and that they are only properly developed in association with a functional lateral line sensory system and a functional tail motor system. In the fishes the development of these fibers is related to the activity and coordination of the swimming movements carried out by the trunk and tail. They are reduced or absent in forms which live on the bottom and in forms without tails.

Graham and O'Leary (1941) studied the spike potentials which appear in the spinal cord of the catfish, *Ameiurus*, following electrical stimulation of the medulla. They recorded rates of 50–60 m. per second at 10–15° C. and associated these values with the axons of the Mauthner cells.

The subject has been recently reviewed by Stefanelli (1951).

*ii. The control of electric discharges.* (See also Chap. V.) Albe-Fessard and Martins-Ferreira (1953) investigated the action of the nervous system in synchronizing the functioning of the numerous electroplaxes of the electric eel, *Electrophorus electricus*. They obtained two main results from their experiments: firstly, the delay in intrametameric spinal transmission of nervous control is not the same at all levels but decreases in passing from the anterior to the posterior end of the animal; secondly, the delay within a given metamere can be reduced by increasing the intensity of the central afferent discharge, although this applies particularly to the more anterior region of the electric organ. Since all the peripheral electroplaxes are stimulated to discharge almost simultaneously, some kind of chronological compensating mechanism must exist. Albe-Fessard and Martins-Ferreira suggested that since the density of the electroplaxes is much greater in the anterior parts of the electric organ and since the number of electro-effector neurones seems to be directly related to that of the electroplaxes, the density of the neurones innervating the electroplaxes must be much greater in the anterior part of the spinal cord than in the posterior part. It is therefore possible, for the simple reason of spatial organization, that fewer fibers tend to converge on each anterior effector-neurone. As a result of this, even a maximal efferent discharge would develop a weaker synaptic potential gradient and a longer latent period at more anterior levels. In the case of a non-maximal discharge the delay resulting from this slow development of the state of excitation would be differentially increased. This would explain the accelerating influence of an augmented intensity of stimulus. So far there is not enough histological evidence to support this hypothesis.

### 4. REGENERATION IN THE SPINAL CORD

Koppányi and Weiss in 1922 (further reported by Pearcy and Koppányi, 1924–1925) carried out spinal section at a high level in *Carassius carassius*. The fish were paralyzed after the operation and lay for weeks practically motionless on the bottom of the aquarium. Later they began to move their fins again and after two months, according to the earlier communication, could swim so well that they could not be distinguished from normal animals. Moreover, the dark color which the fishes assumed after spinal section also disappeared after two months (see p. 98). (According to the later communication, however, the animals always lay on their sides and seldom swam in the normal position.) Koppányi and Weiss considered that there had been functional recovery of the spinal cord and reported that histological examination showed regeneration of the nerve paths which, in most cases, resulted in the reappearance of normal connections.

Pearcy and Koppányi (1924–1925), working with large goldfish (*Carassius auratus*), cut the entire vertebral column in two with scissors so that no bony continuity remained between the regions anterior and

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posterior to the section. During the first six weeks swimming movements were only occasionally observed in the hind part of the fish but after about ten weeks the movements of the hind part were much stronger and swimming was well coordinated. When at rest the fish lay on their sides but they took up a normal position when swimming. Pearcy and Koppányi assumed a functional recovery of the spinal cord as a result of regeneration but did not report any histological examination to support this claim. Further, any remarks about "well coordinated swimming" as evidence of functional nervous continuity should be treated with reserve unless they are very fully substantiated (p. 66).

Hooker (1930–1931) cut across the spinal cord in small goldfish (*C. auratus*) at the anterior level of the dorsal fin. All his fishes were able to swim voluntarily after the operation but when not swimming some remained erect while others lay on their sides. The fact that Koppányi's fish all lay on their sides when not swimming may be explained by the observation of ten Cate (1935) that it becomes progressively more difficult for a spinal fish to hold its body in the normal position the further forward the section is made. According to Hooker, the swimming of his spinal fish was brought about by the pectoral fins and the body musculature of the anterior end of the animal. The tail was not used, although its fin was often passively spread by water currents created by the pectoral fins and gave the impression of voluntary motion. Hooker's goldfish did not live for more than twenty-four days and no histological evidence for regeneration was found at the end of this time. These results could not therefore be considered as contradicting Koppányi's conclusions. In other experiments Hooker (1932) cut through the spinal cords in young specimens of the guppy, *Lebistes reticulatus*, less than four days old. He claimed full coordination and integration of movements accompanying the reestablishment of nervous connections between the two halves of the body in little more than four days after the operation.

More recently Kirsche (1951) carried out spinal section in adult specimens of *Lebistes reticulatus* and reported morphological and physiological regeneration of the spinal cord. Kirsche distinguished various phases during the course of regeneration. The first phase, apparent about four days after the operation, consists of a disorganized growth from the severed stumps. The second phase begins about seven days after the operation with a mitotic increase of the ependymal cells to form indifferent neural cells which then, in their turn, develop into neuroblasts and glioblasts. Further differentiation leads to the formation of normal ganglion cells in both the proximal and the distal stumps. From these cells orientated fibers grow out and neurilemma cells which have arisen from neuroblasts become secondarily associated with these fibers. The growing nerve fibers reach the end of the opposite stump in about twelve days but there is not yet any functional continuity. Finally, about fifteen days after the operation, there is evidence for both morphological continuity and functional recovery. Kirsche suggests that the fact that such a regenerative process does not occur in the spinal cord of mammals is associated with a high degree of differentiation of the ependymal cells so that they no longer possess embryonic potentialities and are incapable of giving rise to new neural tissues. In the fishes, on the other hand, the embryonic potentialities are still present and regenerative processes can accordingly take place.

## C. The Part Played by the Medulla Oblongata and the Spinal Cord of Fishes in the Nervous Control of Muscular Movements and Locomotion

### 1. Introduction

In the nervous control of locomotion and of movements generally the spinal cord clearly plays an essential part. Many investigations have been concerned with reflex motor activity, others with the locomotion of the animal as a whole, particularly with regard to the coordination of body and fin movements, the conduction of locomotory movements along the body, the spontaneity of action of the spinal cord, and the initiation of locomotion.

While the account given here is restricted of necessity to the fishes, the nervous control of locomotion can only be studied properly as a general problem of the vertebrates, investigations on one class giving pointers to the others. For a broader treatment the reader is referred to Gray (1950) and to the various papers by Gray and his collaborators which are in the Journal of Experimental Biology from 1940 onwards.

The subject of the mechanics of locomotion of fishes also lies outside the scope of this chapter. Reference may be made to the papers, with their bibliographies, by J. Gray and by Harris in the Journal of Experimental Biology and in the Proceedings of the Royal Society of London, Series B, from 1933 onward; by Harris (1936); by Breder (1926); and by Breder and Edgerton (1942).

Reference may also be made to Bethe (1941a,b) and to Hebb (1949).

### 2. Unisegmental Reflexes

Ten Cate (1934) cut the spinal cord of the dogfish *Scyliorhinus* in order to isolate a single segment with a pair of dorsal and a pair of ventral roots. The adjacent regions of the cord were removed. He found that mechanical stimulation of the region of the skin innervated by the single intact segment was followed by local reflex muscular contractions. He thus showed that reflex movements could be mediated by a single spinal segment. Similarly he obtained unisegmental reflexes in the pectoral fins. He also destroyed the dorsal root on one side and the ventral root on the other and was able to obtain a crossed unisegmental reflex. In other experiments he isolated a ventral pair of spinal nerves and a pair of dorsal nerves immediately anterior and posterior to them, as shown in Fig. 17. On stimulating the skin innervated by one or the other dorsal nerve ten Cate found no significant difference in muscular reaction, i.e. the motor centers of the isolated pair of ventral nerves were affected in the same way by stimuli entering by either the more anterior or the more posterior dorsal nerves. Ten Cate concluded that in these fish there is no strict division of the spinal cord into segments and that one can only speak of unisegmental reflexes within the limits indicated by these experiments.

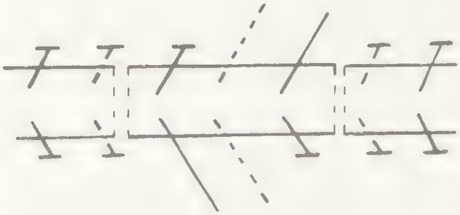

FIG. 17. Diagram to show the method of isolating a part of the spinal cord in *Scyliorhinus canicula*. One pair of ventral roots and two dorsal roots are intact; all the others are cut. Broken lines = ventral roots; continuous lines = dorsal roots (from ten Cate, 1949b).

It would be unjustifiable to conclude that the sum of such reflex units can give a picture of the locomotory activity of the intact spinal cord. It has been suggested that purely segmental activity may be concerned more particularly with trophic functions while the plurisegmental reflexes play the important part in the control of locomotion, the spinal cord clearly acting as a unified structure (von Buddenbrock, 1953, p. 342).

## 3. PLURISEGMENTAL REFLEXES

*a. Experiments on elasmobranchs.* Van Rijnberk (1905a, 1906a) made experiments on the dogfish (*Scyliorhinus catulus*) in which he cut through a few dorsal roots or merely exposed the spinal cord. As a result of this slight damage the preparation carried out sinuous swimming movements which could continue for days. When such a swimming dogfish collided with the aquarium it often became motionless for some time. Slight pressure on the lower jaw also inhibited these swimming movements. In other experiments van Rijnberk attached a wooden clamp to the pectoral fin of an intact dogfish. This was first followed by defense reactions and then by sinuous swimming movements which, again, could be inhibited by touching the lower jaw and other parts of the body.

Frédéricq (1922) described experiments in which he cut off the tail of a dogfish (*S. canicula* and *S. catulus*) between the anal and tail fins. The tail made sinuous movements when it was laid on its side on a table or held ventral side upward but not when it was held in a normal position so that it touched the ground. Bauer (1926) repeated Frédéricq's experiments, describing the movements as quite unsuitable for correcting the abnormal position. The stopping of these movements when the tail, in its normal position, touched the ground was explained by Bauer as a reflex inhibition of the motor centers of the tail from skin sense organs. After spinal section restless swimming movements began. These could be inhibited by gently touching certain regions of the body, particularly those which touch the ground when the animal is at rest. Touching of other regions (e.g. the tail or the tip of both dorsal fins) could result in a strengthening or a release of these movements. After treatment of the skin with cocaine both these reactions disappeared. Bauer therefore concluded that in both cases he was concerned with the stimulation of skin sense organs. Increasing the water pressure on the spinal animal resulted in defense movements followed by a cramp-like contraction. This reaction persisted after the application of cocaine and he therefore thought it was the result of stimulating the muscle proprioceptors. According to Bauer, in the normal dogfish the motor centers in the spinal cord may be inhibited by the skin sense organs, the muscle proprioceptors and the brain.

Craw (1927) investigated reflex activity in the spinal cords of two elasmobranchs (*Raja erinacea* and *R. radiata*) after spinal section immediately behind the medulla oblongata. She used mechanical stimuli to map out definite regions of the skin which could be associated with specific reflex movements of the fins and tail. The extent of these reflex movements depended, within limits, upon the strength of the stimulus. Craw also isolated regions of the spinal cord by means of two transverse sections. In such a region, including the pelvic fins, she was able to obtain both unilateral and crossed reflexes. After cutting through one side of the spinal cord between the last root innervating the pectoral fin and the first root supplying the pelvic fin, Craw found that stimulation of the hinder end of the pectoral fin of the operated side was followed by reflex movements of the pelvic fin of the same side. She concluded that the impulses concerned in this reflex must follow paths which cross from side to side within the spinal cord.

Ten Cate (1933) investigated reflex activity in the spinal cord of the dogfish (*Scyliorhinus*). He found that very weak tactile stimuli applied to spinal fish lying on the bottom of the tank may result in approach and even of contact of the body with the source of the stimulus while a sudden and stronger tactile stimulation may be followed by the removal of the stimulated part of the body. Still stronger stimulation may be followed by beating of the tail and sinuous body movements or, if the stimulation is strong enough and is applied for a sufficiently long time, by actual locomotion. Ten Cate also investigated various fin reflexes. Thus, if the dorsal fin is gently stimulated on one side it bends toward the other side while if the fin is pinched it moves to and fro. Light stimulation applied to the edge of the pectoral fin results in a raising of the fin while if the base of the fin is stimulated it is drawn under the belly of the fish.

Other studies of a similar type were made by Le Mare (1936) who observed the part played by the various fins in the swimming of the shark *Scyliorhinus catulus* and also examined the reflex responses obtained on stimulating specific regions of the body in the spinal animal.

Gray and Sand (1936b) further investigated the reflex activity of the spinal dogfish (*S. canicula*). When a dogfish whose cord has been transected behind the medulla is held by its snout in a horizontal position in sea water it maintains a persistent regular undulatory rhythm at a frequency of about 40 per minute, the rate remaining very constant for any one individual. It may, however, be increased or reduced by the application of suitable stimuli. Thus tactile stimulation applied to any part of the body behind the level of section causes increased amplitude and frequency of the movements. The regions of the pelvic and anal fins appear to be most sensitive in the initiation of this reflex. A wooden spring clamp fixed to the trunk behind the dorsal fin to cause strong pressure produces violent writhing which is soon followed by cessation of movement, the fish lying still, usually bent on one side or the other, with the muscles fairly strongly contracted. Later they relax and the fish lies limp. Strong pressure, e.g. pinching the fins with a clip, causes an increase of amplitude and a reduction of frequency. Unilateral tactile or clip stimulation is accompanied by bending to the stimulated side, the undulatory rhythm becoming asymmetrical in consequence. These reflex phenomena are not properties of any particular level of the spinal cord but may be demonstrated, for example, in the tail piece of a fish whose cord is transected some distance posterior to the level of the cloaca. Such a preparation still exhibits a spontaneous persistent rhythm.

Lissmann (1946a) investigated the effects of deep pressure and of nociceptive stimuli on spinal dogfish (*Scyliorhinus canicula* and *Squalus acanthias*). The application of deep pressure is followed first by a rhythm of greater amplitude but lower frequency than that shown normally by the spinal animal and then, often only after several minutes, by cessation of movement, this cessation having the appearance of fatigue. Lissmann considered that this response was the composite picture of a number of events caused by simultaneous but differential contractions of the myotomes at the same level. He suggested that one might assume reinforcement to take place in response to isometric conditions. Further, preparations which are inhibited through ventral touch, when subjected at the same time to strong pressure, show the same succession of reactions as preparations freely suspended in water. On release there are accelerated and augmented movements followed by a period of swimming beats, despite the continuous ventral contact. If a nociceptive stimulus is applied at the same time as strong pressure, the frequency of the original response is increased, although it is not as high as it is after the application of the nociceptive stimulus alone to an otherwise undisturbed dogfish. The frequency seems to be, as it were, the mean of those associated with the two separate responses (see von Holst, 1934c) (p. 62). Lissmann considered the possibility that the rhythmic component of the response which appears on applying strong pressure might be caused by a nociceptive stimulus acting simultaneously with another stimulus which tends to arrest or retard the movement. If two different sensory mechanisms are involved in this response it may be intelligible that after fatigue of one of them the response emerges in a different form. Thus, after a strong pressure clamp has been repeatedly applied the nociceptive elements may drop out and the response will then only be arrest of the movement. This type of reaction has been recorded by Gray and Sand (1936b).

These rhythmical movements of the spinal dogfish will be considered later in another connection (p. 80).

*b. Experiments on teleosts.* Pflüger (1853) cut through the spinal cord in the eel (*Anguilla anguilla*) immediately behind the medulla oblongata and described various reflexes shown by the posterior portion. Thus he found that the tail or the side of the body was drawn away when a flame was placed near it; the preparation, when placed on its back, would turn over on being stimulated; even when beheaded it could still crawl. Vulpian (1866) cut an eel into several pieces and reported that each portion could carry out movements on stimulation of the skin as long as the spinal cord was intact. Bickel (1897a,b; 1900), using two teleosts, the bleak (*Alburnus alburnus*) and the tench (*Tinca*), cut the spinal cord near the medulla and showed that pinching the tail or removing the fish from the water was followed by lively to and fro movements of the body.

In more recent years there have been a number of detailed studies. Von Holst (1934b) studied some reflexes in spinal teleosts, using *Carassius auratus*, *C. carassius* and *Cyprinus carpio*. After spinal section the animal was left for three days, in which time the effects of spinal shock had disappeared. It was then held firmly by means of a clamp attached to its dorsal fin and movements of the tail were recorded while the fish was stimulated by a jet of water directed onto various parts of the spinal region. After the spinal section only the anterior part of the animal shows rhythmical swimming movements, the hind part being motionless excepting when stimulated.

Von Holst observed various reflexes in spinal goldfish (*C. auratus*) in response to stimulation of specific areas. Thus, in addition to fin movements, he described the "propeller reflex," a rhythmical swinging of the tail fin from side to side in which the lower edge leads; the "fanning reflex" ("Wedelreflex"), a rapid to and fro motion of the hinder edge of the dorsal and anal fins; the "trunk-beat reflex" ("Rumpfschlagreflex"), a powerful beat of the hinder part of the animal against a current directed onto it. This last reflex can be elicited by stimulating any part of the body but the sensitivity of the various regions in this respect varies greatly. The region which moves extends caudally from the part stimulated. Von Holst showed that the sensitivity of the skin in the spinal goldfish is greater than that of the lateral line so that the trunk-beat reflex can be called forth without any response on the part of the body anterior to the point of spinal section. At the same time, the sense organs which are responsible show a very rapid accommodation so that the strength of the current stimulus must rise quickly if the reflex response is to follow. With a constant rate of rise in stimulus strength the strength of the reflex depends closely upon the strength of the stimulus. After some period of stimulation after-discharges appear in the form of beats towards the stimulated side. These were interpreted by von Holst as the result of central summation. Subthreshold stimuli may be summated in this fashion. If the current stimuli are over threshold strength and of sufficient frequency, fatigue sets in. This is limited to the stimulated side, a minimal stimulation of the other side calling forth a normal reflex response.

The above account of the trunk-beat reflex applies to fish with spinal section in the midbody region or more posteriorly. The picture changes as the section is made further and further forward. If the cut lies in the region of vertebrae 7–11, stimulation, particularly by a strong current, results in a beat which is followed by a small but active beat in the other direction. If the cut is almost at the level of the posterior limit of the medulla this second beat is more powerful and is usually followed by a few diminishing rhythmical beats. With section a little further forward these rhythms become stronger while the first beat of the trunk against the current stimulus becomes smaller. Finally, if the cut lies in the anterior region of the medulla at about the exit point of the first vagus root, the stimulus is always followed by a series of increasing and then decreasing rhythmical movements. These results are shown in a schematic form in Fig. 18. They indicate that the ability to react rhythmically lies in the medulla and the adjacent region of the spinal cord and gradually disappears as the cut is made further and further back. (It is not clear from von Holst's account whether the stimulus was always applied at the same place throughout these experiments).

Finally von Holst investigated the effect of current stimuli on the fish with an intact central nervous system after blinding it and cutting the lateral line nerves. He found a great variety of responses and thus demonstrated once again the limitations of the reflexes and their machine-like qualities after disconnecting the brain.

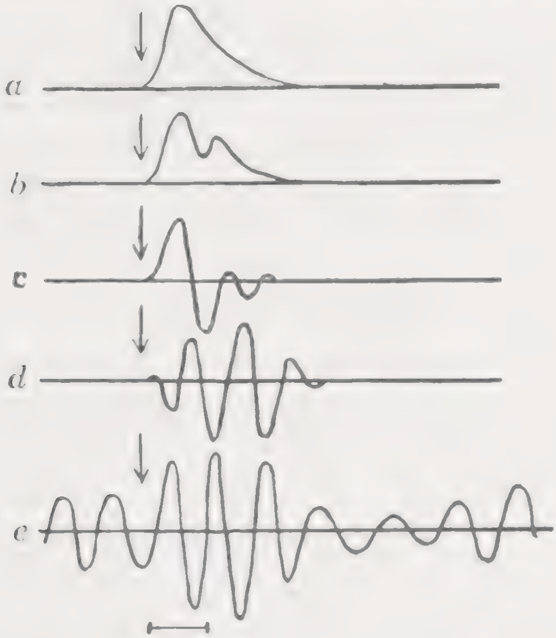

FIG. 18. Diagram showing the form of the trunk-beat reflex after spinal section at various levels. *a:* section in the middle (ca. vertebra 16) or posterior to this point. *b:* behind vertebra 7; *c:* between vertebra 7 and the beginning of the medulla. *d* and *e:* in the anterior region of the medulla (in front of the exit of the vagus). The arrows indicate the stimulating current of water. The line at the bottom indicates the duration of the stimulus (from von Holst, 1934b).

Von Holst (1934c) performed similar experiments on two other teleosts with different shapes of body and arrangements of fins: the eel (*Anguilla anguilla*) and a loach (*Misgurnus fossilis*). He did not find the propeller and fanning reflexes after spinal section but observed a very marked trunk-beat reflex. In these elongated fishes this reflex is in some respects not quite the same as in the goldfish. It takes the form of an S in the entire spinal portion of the animal, the position and form of this S depending upon the place of stimulation. Von Holst showed that the level of spinal section plays practically no part in determining the reaction to mechanical stimulation. Thus, the reaction is the same when the spinal hind portion is anything from $\frac{1}{5}$ to $\frac{2}{3}$ of the total length. The point at which the stimulus is applied is not related in any fixed anatomical manner to the response but only with respect to its relative position in the spinal preparation. This is shown in Fig. 19. In I the spinal section is in the middle of the animal. In II a second spinal section has been made half way along the first spinal preparation. When the point of stimulation bears the same relation to the total length of the spinal preparation, each short piece behaves in the same way and in practically the same way as the original piece of double length. Thus, for example, the anterior piece in II*c* is stimulated in a region which previously, in I*a*, gave another response. Now, however, stimulation of this region is followed by the same response as was given by I*c*. Von Holst therefore concluded that the "middle" or the "end" in such a preparation is not an anatomically determined region but one associated with the state of central nervous balance which exists at the time.

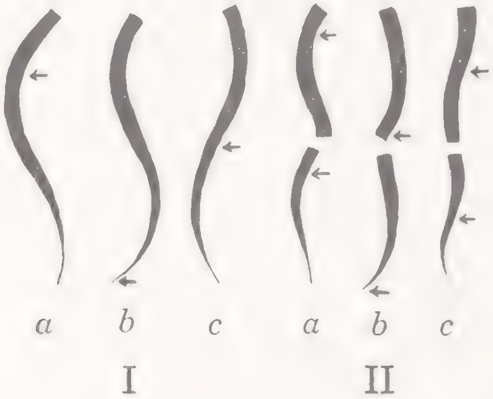

FIG. 19. Diagrams to show the positions taken up by a spinal eel after being touched once by a brush on various parts of the body: I: after spinal section about half way along the body; II: after a second spinal section in the same animal (from von Holst, 1934c).

The trunk-beat reflex in the eel results from a *single* touch on the side with a brush. If the eel is stroked simultaneously on each side with two brushes toward the anterior end of the body and if the excitation is sufficient, the response consists of a number of rhythmical locomotory movements. Also, if unilateral stimulation is strong enough, the bending which results appears to stimulate the other side so that there is, in effect, again a bilateral symmetrical stimulation. The response to this is, once more, in the form of rhythmical locomotory movements. Von Holst concluded that the central nervous discharges only appear to become rhythmical when the stimulation is balanced.

The ability to produce rhythmical locomotory discharges, which is associated in the goldfish with the anterior region of the spinal cord and gradually disappears posteriorly, is thus associated in the eel with regions much further back.

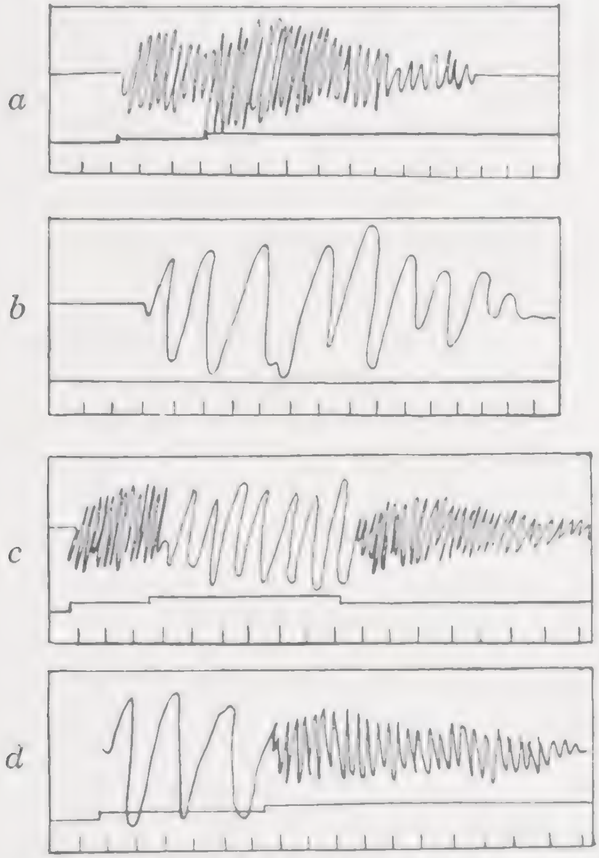

FIG. 20. Eel with spinal section half way along the body, the anterior part of the animal was fixed; a lever was connected at the end of the first quarter of the spinal portion. *a:* a pointed clamp was fixed at about the beginning of the last third of the spinal portion (1st signal) and 15 seconds later a second similar clamp was placed directly behind it (2nd signal). *b:* a flat pressure clamp was placed at about the middle of the spinal portion. *c:* first a pointed clamp was put on (1st signal) and then a pressure clamp (2nd signal). The latter was then removed (3rd signal). *d:* first a pressure clamp was put on (1st signal) and then a pointed clamp (2nd signal). Time trace in 5 second intervals (slightly modified from von Holst, 1934c).

Von Holst studied some aspects of the rhythmical discharges which are produced by the spinal eel some weeks after operation. The form and frequency of the sinuous locomotory movements depend upon the strength of the stimulus and the kind of stimulus. Thus von Holst took a spinal eel which was reacting well and attached a clamp of fine pointed steel wire about the middle of the spinal region. As an immediate response there appeared rhythmical swimming movements of about normal frequency which lasted for some time and only diminished with the onset of fatigue and accommodation (Fig. 20*a*). If the stimulus was increased by placing a second clamp behind the first, there was a clear summation of the stimuli. If, instead of a pointed clamp, one with a large flat gripping surface was used, there were again rhythmical sinuous movements but now they were much less frequent, each one lasting longer and being more powerful than before (Fig. 20*b*). When the flat clamp stimulus was increased beyond a certain strength the movement became steadily less and, when the pressure was very great, was completely inhibited. Biologically, according to von Holst, the pointed clamp signifies "swim away" and the flat clamp "wriggle out." The intact eel shows these reactions clearly enough. If it is held in the hand the wriggling movements become slower and more powerful the harder the animal is gripped. If the grip is very tight the wriggling stops and other reactions appear, e.g. biting. In an experiment recorded in Fig. 20*d* the flat pressure clamp was applied and then, 28 seconds later, the pointed clamp was placed immediately behind it. The amplitude diminished and the frequency increased. Usually (von Holst), although not very clearly in this record, the frequency is less than when the pointed clamp is applied alone. Conversely, in Fig. 20*c*, when the flat clamp was applied 13 seconds after applying the pointed clamp, the resulting frequency and amplitude lay between the values obtained by either type of stimulus alone. After removing the flat clamp the original rhythm was observed again. It thus appears that the result of applying both types of stimulus together is something intermediate between the results which are obtained when the two stimuli are applied separately. When the two stimuli are not applied simultaneously but a short time after one another, as in Fig. 20*c,d*, the effect of the second stimulus is more pronounced than that of the first. The mechanisms underlying these reactions were not investigated by von Holst.

Von Holst further showed (1934c) that the spinal eel, without the obvious application of any stimulus, may carry out rhythmical side-to-side swimming movements. This rhythm is usually very regular although there may be occasional periodic increases in amplitude and frequency. Such a rhythmically moving preparation can show reflexes, just as can the resting spinal preparation. Thus the attachment of a pointed clamp results in a persistent increase of the rhythm while a flat gripping clamp causes a slowing-up. The place where the clamp is applied also affects the response: the further forward it is, the clearer is the inhibition. A sufficiently strong pressure clamp at the anterior end stops movement at once; when the clamp is taken away the movement goes on as before with the same amplitude and frequency. This inhibiting of locomotory movement was compared by von Holst with the fact, discovered by Sherrington, that the rhythmical movements of the freely hanging hind leg of the spinal dog can be inhibited by placing the foot upon a solid substratum. As soon as the foot is no longer in contact with this the motion starts again as before.

Von Holst concluded that a distinction could be made between an excitatory or an inhibitory stimulus whose effect persists after it is no longer applied and a stimulus which does not have a persistent aftereffect. The stimulus of a strong pinch belongs to the former category, the stimulus of the flat pressure-clamp to the latter. While these differing responses are difficult to explain physiologically, their biological explanation is clear (von Holst): the persistent movements following a pinch serve for escape while the "wriggle out" movements have no point after the animal is out (see p. 56).

Gray (1936a), working on the eel, also showed the formation of a localized contraction as a reflex response to a gentle touch. Further stimulation of the same region is followed by an increased degree of contraction and an extensive contraction on the contralateral side of the body anterior to the stimulated region (see von Holst, 1934c). This secondary contraction develops slowly and dies away slowly after the source of the stimulus is removed. Gray pointed out that the most significant feature of the reflex is the development of the contralateral contraction which follows an ipsilateral contraction which is itself the reflex response to tactile stimulation. This response is normally of a static postural type but persistent and relatively intense stimulation is followed by active forward swimming movements. According to Gray, "The initiation of swimming in the spinal eel in response to persistent exteroceptive stimuli appears to resolve itself into two phases: firstly, the development of a state of undulatory tone which throws the body into a wave-like form and, secondly, the transmission of this state of tone posteriorly over the body of the animal."

Gray (1936b) showed that when an intact eel inside a glass tube is progressing backwards by means of waves of contraction propagated towards the anterior end of the body, as in normal reversed swimming, the pattern of response changes abruptly as soon as the tip of the tail comes into contact with the edge of the glass tube. When this happens the tip no longer initiates bilateral waves of contraction but curves sharply towards the side in contact with the glass and the eel moves rapidly out of the tube by means of a wave of muscular contraction which passes anteriorly along the side of the body which had come in contact with the edge of the tube. Similar phenomena are observed in a reverse direction when an eel moves forward out of a tube. These posterior and anterior resistance reflexes can also be elicited from spinal and decapitated preparations.

Von Holst and his collaborators, in other studies, have described the body and fin reflexes in a number of teleosts. Thus von Holst and Le Mare (1936) determined the responses to stimulation of various regions of the body in *Crenilabrus*, *Sargus*, and *Uranoscopus* in an attempt to find the plan of the locomotor reflexes. They found a distinction between reflex movements which can be obtained in the spinal animal and those obtained in the normal animal. Thus, in the spinal animal, they were unable to elicit swimming backwards and certain other movements of the pectoral fins shown by the intact fish and they assumed that these were associated with the activity of higher regions of the brain. Further, they observed a change in the reactions and in the whole excitability of the animal with varying levels of spinal section and the appearance of new reflexes on progressive shortening of the spinal cord. The same stimulus could result in reflex movement when spinal section was at one level but in inhibition of movement when the section was at another level. Section through the anterior region of the medulla oblongata was followed after a short time, without stimulation, by rhythmical swimming movements of the pectoral and dorsal fins. These movements were also seen if the cut was further back but a longer time after the operation elapsed before they appeared. These movements were made slower or were stopped completely through touch or pressure on the sides of the body, on the belly, or on the anal or pelvic fins while, if the spinal cut was still further back, these movements only appeared in response to similar stimulation of anal and pelvic fins. Fig. 21 shows typical responses obtained from the spinal *Labrus-Crenilabrus* type. The results obtained from other types are presented in later publications (Eberhard *et al.*, 1939).

Experiments of this nature were also made by Tuge and Hanzawa (1935), working on *Oryzias latipes*, *Carassius auratus*, and *Cyprinus carpio*. When spinal section was carried out without an anesthetic complete shock only lasted about one minute. The reflexes then gradually returned. These workers mapped out some of the cutaneous areas in the spinal fish which would elicit specific reflexes on stimulation.

Some of the above results recall an earlier description by Bauer (1926) of an inhibition which he observed in the giant goby (*Gobius capito*). In this fish the pelvic fins form a sucker which enables the animal to attach itself firmly to rocks in all possible positions, not infrequently with its head pointing upwards or downwards. When the fish is not attached its pectoral fins take part in reflex movements which help to maintain the animal in its normal position of equilibrium for swimming. If, however, the pelvic fins are touched, either by an observer or because the fish is attaching itself to a rock, these reflex movements are inhibited and

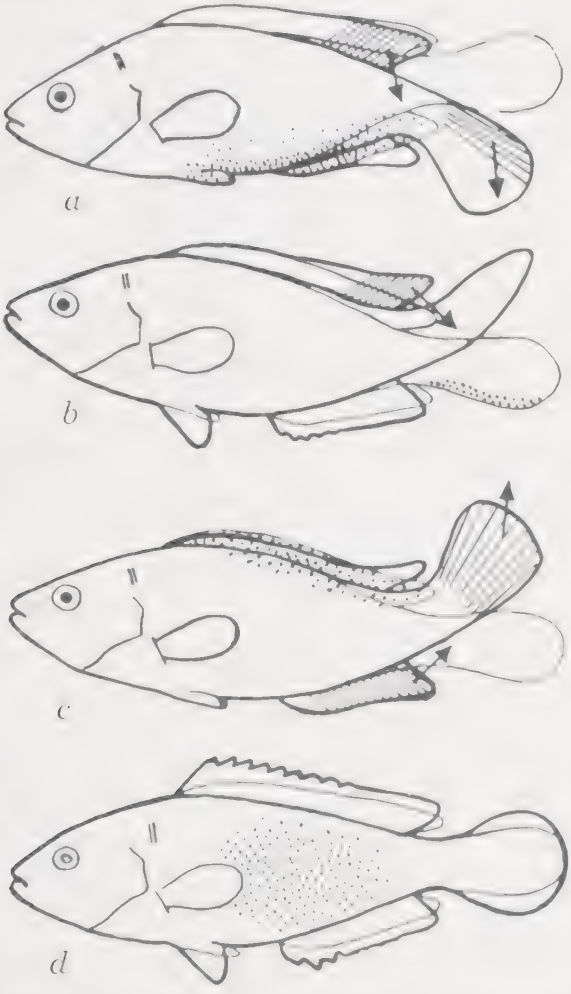

FIG. 21. *Labrus-Crenilabrus* type: *a:* reaction to tactile stimulation of the ventral region (shaded with dots); the parts of the fins which show active movement are indicated by drawing the fin rays while the direction of the current of water produced is indicated by an arrow. The initial position of the animal is indicated by thin lines and that taken up on stimulation by thick lines. *b:* reflex following stimulation of the ventral tail region; *c:* following stimulation of the back. *d:* reflex spreading of the fins (from von Holst and Le Mare, 1936).

the pectoral fins are carried in a fixed position, spread out to the side of the body.

(In connection with this section, reference may be made to a survey of segmental reflex activity in the spinal cord by ten Cate (1949b).)

## 4. The Conduction of Locomotory Movements along the Body

The regular passage of waves of movement along the body of a fish during locomotion was at one time thought to be brought about by a series of chain reflexes. This belief was supported by the work of Friedländer (1894) who had cut through the ventral nerve cord of the earthworm and shown that the posterior part could carry out typical peristaltic locomotory movements purely through the mechanical pull exerted on it by the anterior region. Friedländer concluded that the contraction of one segment stretched the next one and so excited its proprioceptors; the resulting reflex contraction, in its turn, excited the proprioceptors of the next segment, and so the wave of contraction passed down the body of the animal from segment to segment as a so-called "chain reflex."

In connection with this theory of chain reflexes numerous experiments have been made on locomotion in fishes. Among earlier workers Bickel (1897a) cut across the spinal cord in the eel (*Anguilla anguilla*) at a point about one-eighth along the body from the head and observed that the posterior portion behaved like a decapitated eel, carrying out spontaneous sinuous movements by which the anterior part was also moved passively. Occasionally the anterior part initiated locomotory movements which stimulated movements in the posterior part. This was clearer when the spinal section was made further back: the anterior part moved actively and drew the posterior part after it, the sinuous movements of the anterior portion being reproduced in the posterior portion so that Bickel could not distinguish the swimming preparation from a normal eel. In other experiments Bickel destroyed the posterior half or the middle third of the spinal cord and found that the swimming movements appeared over the entire animal. He could only distinguish the operated animals by the lack of tone of the muscles in the region where the cord had been destroyed. Bickel also cut an eel in half and then sewed the two halves together; again the posterior half followed the movements of the anterior half. He concluded that the movements of the anterior half of the spinal animal can stimulate the posterior half to carry out locomotory movements in the manner suggested by Friedländer but that passive mechanical transmission of the movement can also take place, this latter occurring in the partially pithed preparation.

Ten Cate (1933) compared the locomotory movements of the posterior region of the spinal dogfish (*Scyliorhinus*) with those of the same region after destruction of the cord behind the point of section. He decided that the movements in the latter case were much the weaker and concluded that the sinuous locomotory movements of the posterior portion with intact spinal cord were brought about actively through the central nervous system while the weaker movements of the partially pithed preparation were purely passive. Spinal section did have some effect upon the swimming of the dogfish. While the normal animal swims chiefly by the sinuous movements of the caudal region, after spinal section in the middle of the body he maintained that these movements were stronger in the anterior region.

Following other experiments, ten Cate and ten Cate-Kazejewa (1933) reported that after cutting through the spinal cord and all the musculature of the dogfish at the level of the pectoral fins and leaving the two parts of the fish connected only by skin and entrails, the posterior part still showed active swimming movements coordinated with those of the anterior part. If, however, the spinal cord was not only cut but removed for about five segments they found that the coordination between the anterior and posterior parts was weakened. From these results they believed that chain reflexes, as suggested by Friedländer for the earthworm, were playing some part in the passage of the contraction from segment to segment but that, in addition, the overlap of the dorsal spinal nerves (p. 47) allowed sensory impulses to pass directly from the contracting segment itself to neighboring segments, i.e. that there was a direct nervous transmission of the excitation from segment to segment.

Von Holst (1935a) examined the chain reflex theory of fish locomotion experimentally, arguing that if the dorsal roots were cut along the full length of the spinal cord while keeping the ventral roots intact, the possibility of a reflex caused by muscular movements would be eliminated. He carried out an operation of this nature on the tench (*Tinca*), only leaving intact the two most anterior dorsal spinal nerves which innervate the pectoral fins. Despite this, the fish still made swimming movements with its body when it was suitably stimulated on the skin of the gills, head, or pectoral fins or when it was taken out of the water and then replaced. Von Holst concluded that the swimming movement which passes along the body is not brought about by a chain reflex. To support this conclusion von Holst carried out other experiments based on the consideration that if the conduction is of the nature of a chain reflex two conditions must be met: (1) The slow passage of the wave of movement along the body of the animal must be determined through the reflex arcs which follow one another. Therefore, after section of the spinal nerves on both sides over a middle portion of the body, either the reflex chain must be interrupted or the impulses must pass with greater speed over this region, i.e. jump it. (2) The mechanical securing of a middle row of segments must inhibit or, at least, strongly hinder the conduction of the excitation. He used two fishes with long bodies which clearly show sinuous locomotory movements: the eel (*Anguilla anguilla*) and a loach (*Misgurnus fossilis*). After cutting all spinal roots in the middle of the animal and securing this region so that it was held rigidly the innervated anterior and posterior ends of the fish moved in an energetic and fully coordinated fashion. The excitation responsible for these muscular movements appeared to pass along the immobilized region with its severed spinal nerves at a normal or perhaps very slightly reduced rate. Again, in the free-swimming animal operated in this way there was full coordination of movements between the anterior and posterior portions although the middle denervated region was clearly less well coordinated. A similar observation was made when the waves were going in the opposite direction in an eel which was swimming backwards. In another experiment stiff wires were inserted along the sides of the vertebral column in the midregion of the fish so that the segments concerned could not move. The nervous system was left intact. Von Holst did not observe any interruption of the conduction of locomotory movements; while the midregion did not move, the movements of the posterior region had the same relation to those of the anterior region as normally exists in the intact animal. All these experiments thus provided further evidence against the chain reflex theory of locomotion.

Unlike ten Cate (1933) but in agreement with Bickel (1897a), von Holst (1935a) found no difference in swimming between eels subjected simply to spinal section and those in which, after section at the same level, the posterior region of the cord had been destroyed. Free-swimming eels operated in both ways could only be distinguished with difficulty from normal animals (Fig. 22). On the other hand, if the section (with or without destruction of the posterior region of the cord) was made at some point anterior to the middle of the eel, the result was different and actual coordination ceased. Von Holst therefore concluded that even a complete harmony of movement is no evidence for functional activity in the hinder part.

Earlier workers had usually assumed that the wave of movement which passes over the body corresponds to some central process of excitation which spreads over the body with regular velocity. The results of von Holst's work lead him to doubt the presence of any such excitation wave corresponding to the movement wave. He pointed out that the speed with which the visible movement waves pass over the body is very much less than that of the conduction of excitation which is known to exist in the spinal cord. He further observed that in passing from rest to movement the waves form over the whole body almost at the same moment and then proceed backwards together. When the animal stops the waves stop just where they are and do not first all pass to the posterior end. The same applies to an animal with a middle denervated region. Von Holst concluded that all segments are independent of one another and that their speed of movement is influenced simultaneously. He supposed the process to take place in this way: At the beginning of locomotion the segments first assume some moment of the usual movement coordination, i.e. one of the usual S-forms. Thereupon all segments begin to carry out their alternating pendulum movements at the same instant and at the same rate; the *visible* result is the passage backwards of the bending waves over the body. The speed with which they pass over the body is a function of the speed of the rhythm of all segments.

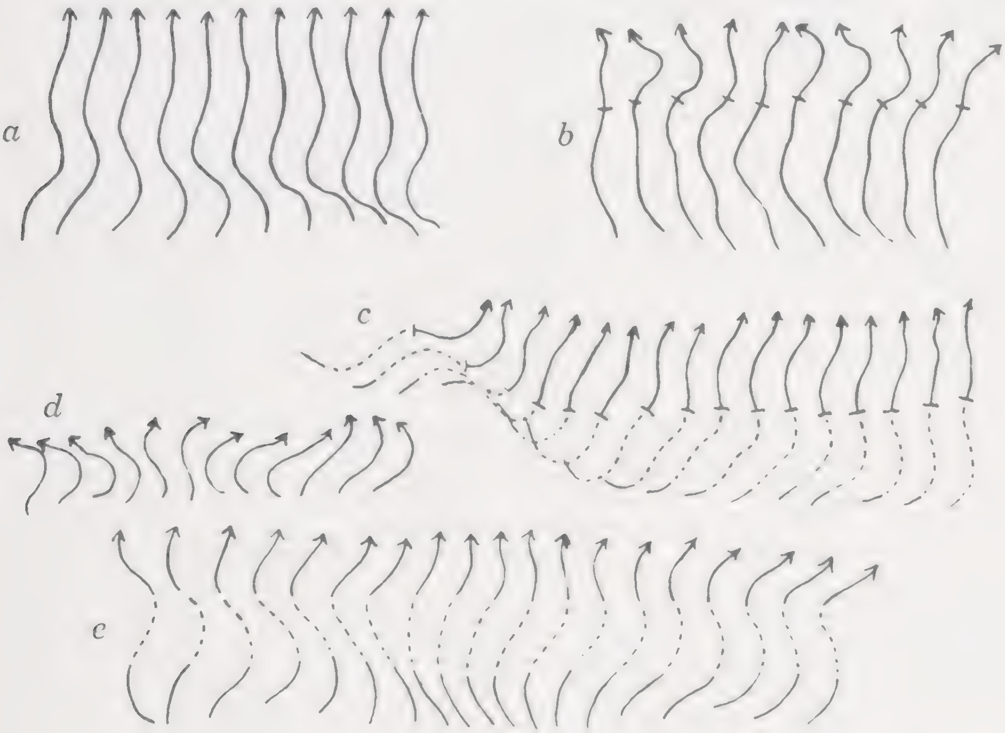

FIG. 22. Diagrams of the motion of an eel under different conditions. *a:* intact animal swimming slowly; *b:* eel with spinal section at the level of the transverse line. *c:* eel with the spinal cord destroyed (excepting for last small portion) behind the point of section. Broken lines indicate destroyed cord. *d:* anterior half of the eel shown in *c* after cutting off the hinder portion. *e:* eel with the roots of the spinal nerves cut through on both sides in the middle region of the body (broken lines) (from von Holst, 1935a).

Further evidence against the chain reflex theory was provided by Gray (1936a) who removed the whole of the skin of the eel and found that it still swam normally after it had recovered from the anesthetic. He concluded that the sense organs of the skin play no essential role in the propagation of muscular contractions along the body of the fish although the possibility remained that nervous impulses arising in the skin might modify the activity of the segmental muscles. Similarly Gray showed that impulses arising in the muscles or in the associated connective tissue did not play a necessary part in the neuromuscular mechanism. He removed all the muscles over a region of 12.5 cm. in a 50 cm. fish and found that after the operation there was no substantial modification of the activity of the posterior part of the body. In order to distinguish between the transmission of an active muscular rhythm and the transmission of a mechanical wave over an otherwise inert region of the body, Gray removed all the muscles for a length of 8 cm. from both sides of an eel immediately behind the anus, leaving two regions of the body connected by the vertebral column. The latter was then held rigid by means of wooden splints wired to it and filling the space normally occupied by the segmental muscles. After recovery from the anesthetic the fish swam normally and, by holding the splints firmly, it could be seen that active swimming movements of the anterior end of the body were unmistakably propagated over the posterior region, only feeble movements of the anterior portion failing to pass over the demusculated region. These facts led Gray to believe it most probable that the transmission of regular rhythmical waves of muscular contraction can occur in the entire absence of peripheral impulses from sense organs in the skin or muscles and is effected by the spinal cord only. In this, then, there is agreement with von Holst (1935a).

Gray (1936a, p. 175) also drew attention to the fact that an intact eel, when at rest, seldom exhibits uniform tone on both sides of the body but almost always lies with its body curved to one side or curved into a wave-like form.When it swims forward the waves which are already present move backward over the body (p. 68); when it swims backward the waves move forward. When movement ceases a wave of contraction passing along the body can stop in any position (see von Holst, 1935a). Gray therefore also believed "that the passage of a locomotory wave over the body of an eel is not the expression of a simple series of excitatory stimuli passing down the spinal cord from the medulla and activating the muscles as it passes." He suggested that an analysis of the activity of the fish's body "might be based on the conception of a definite pattern of posture capable of being transmitted along the body. If the level of postural activity at any one point on the body is upset, definite and coordinated changes appear to be induced at other levels and on both sides of the body." Further, he considered as a strong possibility the interpretation that "there is a relationship between the mechanism which maintains a static state of undulatory posture in the resting fish and that which maintains the difference in phase between successive segments of the freely swimming fish. The response of the spinal eel to unilateral stimulation suggests that a localized unilateral state of spinal activity induces a secondary region of activity lying contralaterally and anteriorly to itself; it is possible that typical undulatory posture is determined in this way. A sustained swimming rhythm would result if one such localized unilateral state of spinal activity were propagated along the body for it would automatically induce the formation of an anterior contralateral wave travelling at the same speed as itself. If this interprepation of the facts be correct, the possibility of eliciting a self-generating rhythm would be dependent on the integrity of an adequate length of spinal cord."

Support for these views was provided by some experiments by Gray and Sand (1936a). They cut the spinal cord of the dogfish (*Scyliorhinus canicula*) immediately behind the medulla and also at a position varying from 12 to 30 segments from the site of the first transection with pithing behind this second transection. Approximately 25 segments of the nerve cord are necessary in order that a spinal preparation may exhibit spontaneous and persistent rhythmical activity in the absence of extraneous stimuli.

Gray and Sand (1936a) pointed out that the conclusions of ten Cate and ten Cate-Kazejewa (1933) with regard to *Scyliorhinus canicula* and *S. catulus* (p. 67) do not agree with those of von Holst (1935a) and Gray (1936a) regarding some teleosts. Gray and Sand found that within a few hours of spinal section every dogfish, when lying on the bottom of the tank, showed a marked and persistent rhythm whose amplitude was rather less than that of the intact fish swimming freely. This activity did not appear to depend upon tactile stimulation, for on vertical suspension of the fish by the head or horizontal suspension by a denervated fin the rhythm persisted. Gray and Sand found it practically impossible to assess the degree of active coordination between two regions of a spinal fish by visual observation since all movements which originate in one region of the body automatically induce apparently coordinated movements in another region, interfering mechanically with one another. Accordingly they recorded solely by cinematography. After complete denervation of all somatic muscles for 12 segments at the level of the anterior dorsal fin in an otherwise intact fish they held the denervated region rigidly in a clamp so that there was no mechanical transmission of movement and observed the intermittent rhythm which is characteristic of the normal fish with complete coordination of the anterior and posterior portions. As soon as the spinal cord was cut coordination broke down. Gray and Sand concluded that the mechanism of transmission of the locomotory rhythm in the dogfish is essentially of the same central type as that of the eel and the tench, as shown by the work of von Holst and of Gray (above), chain reflexes playing no part.

## 5. THE SPONTANEITY OF ACTION OF THE SPINAL CORD AND THE INITIATION OF LOCOMOTORY MOVEMENTS

*a. Theories.* Two main theories have been put forward regarding the mechanisms responsible for the initiation of movements: (1) That there is some automatic mechanism within the nervous system which sends out rhythmical waves of excitation and so initiates locomotory movements, these movements only ceasing when suitable inhibitory stimuli are applied to the animal. (2) That the animal is normally at rest and only assumes locomotory activity under the influence of some excitation.

*b. Some investigations on cyclostomes and elasmobranchs.* Steiner (1888) reported that in spinal cyclostomes the anterior portion continued to make locomotory movements while the posterior part remained still and could only be made to move when stimulated artificially. He believed that the operation had depressed the excitability of a nervous locomotory apparatus in the spinal cord. Ten Cate (1935) considered that the severity of Steiner's operations was responsible for these observations. Ten Cate himself found that, in adult *Petromyzon* which survived similar operations for 2–5 days, the posterior part, as well as the anterior, carried out sinuous swimming movements. He suggested that the spinal cord of *Petromyzon* was able to control the movements of the hind part of the animal but that, in order to maintain its activity, it had continually to receive stimuli, either from outside the animal or from the anterior region.

Steiner also found that headless or spinal elasmobranchs (*Scyliorhinus* and *Torpedo*) could still swim and ascribed these movements to stimulation of the spinal cord by the cut. On the other hand, he also described an experiment in which he removed the anterior part of the medulla oblongata and found that the respiratory movements remained unchanged while locomotory movements stopped, the only response to a mechanical stimulus being a twitch. From this he assumed the presence of a locomotion center in the anterior region of the medulla.

Bethe (1899) rejected Steiner's idea of a general movement center in the medulla oblongata, since he maintained that transverse section at different levels through this part of the brain in *Scyliorhinus* was not followed by cessation of locomotion. On the contrary, after section of the medulla oblongata between the posterior region of the cerebellar peduncle and a point midway between Nerves VIII and IX, he observed regular periods of locomotory movement alternating with periods of rest. The swimming movements began with very weak rhythmical beats of the tail which gradually became stronger so that the animal moved forward. On strong stimulation of the body, for example, by burning, rubbing, or pricking, these movements were inhibited and stopped at once. No flight or defense movements were observed. Weaker stimuli, on the other hand, readily produced reflex movements. Bethe concluded that the operation had not eliminated a general movement center but that it had resulted in an increased susceptibility to inhibition. If the cut was made through the medulla behind the midpoint between Nerves VIII and IX these inhibition phenomena no longer appeared and all stimuli were followed by lively movements. After section of the medulla posterior to Nerve X the preparation carried out persistent locomotory movements and Bethe ascribed these to a permanent loss of inhibition.

As already mentioned (p. 55) Bauer (1926) concluded that the restless swimming of the spinal dogfish could be inhibited by touching certain regions of the body but released or augmented by touching other regions. He considered that motor centers in the spinal cord of the normal dogfish may be inhibited from the skin sense organs, the muscle proprioceptors, and the brain.

Ten Cate (1933) (p. 56) found that sufficiently strong stimuli applied to a spinal dogfish lying motionless on the bottom of its tank were followed by locomotory movements.

Gray and Sand (1936a,b) found a marked and persistent rhythm in the spinal dogfish (*Scyliorhinus canicula*) when lying on the bottom of the tank. This was not dependent upon tactile stimulation, for after suspension of the fish by the snout or by a denervated fin in such a way that the spinal region was isolated from contact with a solid surface, the locomotory rhythm persisted for many hours until observations were discontinued. This rhythm was found to be sensitive both in frequency and amplitude to tactile and nociceptive stimuli. Gray and Sand therefore concluded that the presence of a locomotory rhythm in the body of a spinal dogfish depends upon the general level of excitation of the spinal cord. Above a certain level of excitation a locomotory rhythm emerges. Reducing the level (e.g. by putting the dogfish on its back) abolishes the rhythm. Further, they cited Bethe (1899) as showing that transection of the brain of the dogfish in front of the medulla does not interfere with the normal powers of locomotion. They concluded that the powerful but intermittent rhythm characteristic of the intact dogfish is brought about by the influence of the medulla on the weaker but persistent rhythm which is an inherent property of the isolated spinal cord.

Le Mare (1936) also observed the rhythmical swimming movements of the spinal dogfish (*Scyliorhinus catulus*, *S. canicula*, and *Mustelus "vulgaris"*). In preparations supported by a clamp anterior to the section he reported that movements were accentuated during and after mechanical stimulation and inhibited by holding either of the dorsal fins. On release of the inhibitory stimulus Le Mare observed a post-inhibitory rebound, the first few beats being of abnormally large amplitude. (Lissmann (1946a), using *Scyliorhinus* and *Acanthias* (= *Squalus*), was unable to confirm these types of inhibitory action.) Le Mare further noted that after section of the spinal cord of *Scyliorhinus* at any level, the part of the body posterior to the section carried out rhythmical swimming movements. He pointed out that in some teleosts (p. 58) such movements only occur when the section is made at the anterior end of the medulla and suggested that in these the neurones controlling the movement are probably confined to the medulla while in the dogfish they are dispersed throughout the length of the cord, this representing a more primitive condition.

*c. Some investigations on teleosts.* Steiner (1888) found that the decapitated eel (*Anguilla anguilla*) could swim without interruption for hours at a time. Bickel (1897a) made a similar observation after spinal section at various levels, noting that his animals could only swim forwards. Weak stimuli were inhibitory but strong stimuli increased the locomotory movements. In other experiments Bickel (1900) observed the movements of the tench (*Tinca*) and the bleak (*Alburnus alburnus*) after spinal section at different levels. There were no spontaneous locomotory movements in the portion posterior to the section but Bickel believed that the time between the operation and the observations might have been too short (see the observations of Dijkgraaf, 1949b). Hooker (1930–1931) performed similar experiments on the goldfish (*Carassius auratus*). He concluded that the spinal animal progressed through the water by means of the pectoral fins and that there was no active movement of the region posterior to the section. Polimanti (1912b) considered spinal automatism to be generally much more limited in teleosts than in elasmobranchs. Weber (1927) claimed that after beheading the seahorse (*Hippocampus*) the tail can respond to tactile stimuli by bending towards the stimulated side but the fin movements and steering movements of the tail, normally directed by the brain, no longer appear. However, Weber's animals were subjected to a very gross attack and only lived for 10–15 minutes, a time which, as ten Cate (1935) has pointed out, is too short for reliable observations. In this connection, reference may also be made to the work of von Holst (1934b) (p. 76).

Dijkgraaf (1949b) found that blinded spinal minnows (*Phoxinus*) suspended in water show apparently spontaneous body and fin movements without any obvious stimulation. The first slight movements begin 8–10 days after spinal section at the level of vertebra 6–7 or posterior to that region. When section is near vertebra 1 the movements begin within 2–3 days. In the following days or weeks their intensity increases until the posterior part of the body carries out swimming movements which drive the anterior region passively through the water. Periods of swimming, which may last for 1 minute, alternate irregularly with periods of absolute rest. Often the movements of the posterior portion cause a secondary excitation of the anterior portion. Very seldom movements start in the anterior part and, after what appears to be a latent period of anything up to 1 minute, the posterior portion becomes secondarily excited. There was no question of regeneration of the cord during these observations, as a piece about the length of two vertebrae was removed at the time of the operation and the stumps did not rejoin. The significance of these "spontaneous" movements and the part played by afferent excitations has not been investigated. According to Dijkgraaf, they give the impression of some central automatic process such as was postulated by von Holst (p. 76). It appears as if the state of activity of the spinal cord gradually rises after the operation until it "boils over." When the spinal cord is sectioned at the level of the dorsal fin in a minnow already subjected to spinal section further anteriorly which already shows "spontaneous" swimming, tail movements reappear after 1–2 days. The change in the state of activity of the spinal cord after section thus seems to affect all regions of it to the same degree.

Gray (1936a) agreed that the source of rhythmical locomotory activity in the eel (*Anguilla*) lies in the medulla oblongata or in the spinal cord. After decapitation behind the medulla the eel may exhibit activity for a short time after which the preparation remains inert. It can be induced to swim by the application of appropriate stimuli to the severed end of the spinal cord. Thus, after stimulation with condenser discharges of variable intensity above a critical intensity value, regular forward swimming movements begin. The frequency of these rhythmical contractions is much lower than that of the applied stimuli, there being no direct relationship between the two. If the strength of the stimuli is increased slightly beyond that required to elicit this response, the preparation becomes inactive. Further increase in the strength of the stimuli results in a new rhythmical response in which the locomotory waves of contraction pass the other way, consistent with swimming backward. Within limits, the higher the intensity of the stimulus the greater is the amplitude of the movements. Gray suggested that the normal locomotory rhythm might involve specific activity of the medulla although the stream of impulses being supplied by the medulla is not itself of a rhythmical nature. However, he pointed out that it is possible to elicit rhythmical movements from a decapitate or spinal preparation by purely peripheral stimulation, such as adequate pressure on the tip of the caudal fin.

In further experiments Gray cut the spinal cord immediately behind the medulla or between the 1st and 4th vertebrae. Such an eel lay motionless on its side, in contrast to the observations of Bickel (1897a) (p. 74).

Gray suggested that the activity of Bickel's eels might have been brought about by spinal regeneration. Gray's preparations exhibited well-marked rhythmical movements on mechanical stimulation of the tip of the tail, e.g. by the attachment of a small clip. Such observations indicated that the level of spinal excitation required to produce a swimming response can be provided by peripheral stimulation as well as by the direct application of electrical stimuli to the cord. Cinematograph records showed that the first phase of the response is the development of undulatory tone whereby the posterior end of the fish is thrown into a wave form. The waves then begin to move toward the posterior end of the body and new ones form in front of them (p. 70). Gray summarized by saying that the spinal cord of the eel has an intrinsic activity (inherent locomotory rhythm) "which it only expresses when conditioned by stimuli of either peripheral or central origin. In the latter case the requisite level of excitation is effected by the medulla." (See Lissmann, 1951.)

*d. The case for an automatic rhythmical mechanism, independent of peripheral stimulation.* Von Holst (1934a,b) made a number of investigations on the trunk-beat reflex in the goldfish (*Carassius auratus*) (p. 58). He argued that when the reflex is released to the left and immediately afterwards to the right there should be a normal wave of locomotion. The question therefore arose, whether the currents which result from locomotion can act as stimuli and elicit the rhythmical body movements reflexly, i.e. is an alternating water-current stimulus sufficient to cause locomotory movement? Experiments indicated that it is not, for responses stopped as soon as the frequency of the stimulus which was applied alternately to the right and left sides was less than that associated with normal swimming movements. Von Holst concluded that alternating exteroceptor skin reflexes could not be held responsible for maintaining rhythmical locomotory movements in the intact fish. Further, if stimuli arising from proprioceptors were significant, the bending which follows stimulation on one side in the spinal fish could be expected to produce a proprioceptive stimulation on the other side and so further bending, leading to locomotory movements. In fact, this does not happen. Von Holst only obtained one beat on stimulation and concluded that the proprioceptors also play no part in the initiation of contractions in normal locomotory movements. These negative conclusions suggested the possibility of a rhythm generated automatically within the central nervous system itself. This idea was strengthened by the discovery that the trunk-beat reflex assumes a more and more rhythmical form as the point of section is moved from the spinal cord to the anterior region of the medulla oblongata (p. 58). Von Holst therefore concluded that some rhythm arises in the medulla and adjacent regions of the spinal cord, pointing out that these regions of the central nervous system have been shown in many fishes to control rhythmical functions which have no close relation to one another such as respiratory movements, swimming movements of the body and tail, and movements of the pectoral fins. In the intact animal all these three movements are irregular and independent of one another and respond to quite different stimuli. However, by placing the goldfish in a weak solution of urethane, von Holst was able to demonstrate a close relation between them. As the narcotic begins to affect the animal regular rhythmical swimming movements are seen. These decrease in amplitude as the narcosis becomes deeper and finally disappear. On recovery the reverse takes place, the movements increasing in amplitude and then usually disappearing very suddenly. At the same time, during the onset of narcosis, the respiratory movements also become very regular and suddenly become synchronized with those of the swimming rhythm. In most cases the pectoral fins also develop regular rhythmical movements which are synchronized with the other movements. The *phase relations* of these movements may vary widely and appear to bear no relation to one another: von Holst's emphasis is upon *synchronization*. Thus, for example, variations in the respiratory movements are accompanied by similar variations in the movements of the tail.

Von Holst attempted to estimate which regions of the nervous system had been put out of action by the narcotic when this synchronization appeared. In the first stage of narcosis, during which regular fin movements begin to appear, the stimulus of a current of water directed against the side of the fish is followed by the reaction which is typical of the animal cut in the anterior medullary region (p. 58). Von Holst therefore assumed that in this stage of narcosis the brain was already largely out of action. At the stage of narcosis when the rhythms are synchronized the current stimulus usually no longer produces any reaction. In deeper narcosis, shortly before the rhythmical movements disappear, there is no response to tactile stimuli (excepting touching the gills or pinching the dorsal fin). From these observations von Holst concluded that the synchronization of the three rhythms appears when the region in front of the medulla oblongata is almost or completely out of action and when the reception of excitations from the periphery is greatly reduced or stopped. He considered the stopping of impulses from the periphery to be essential, since unnarcotized animals with a cut in front of the medulla (i.e. separated from the higher brain centers) do not show synchronization of the three rhythms.

These results led von Holst (1934b) to postulate the existence of a single automatic rhythmical process in the anterior region of the spinal cord whose activity represents the common basis for respiratory, pectoral fin, and tail movements. In the absence of peripheral excitation these movements are carried out synchronously. Exteroceptive or proprioceptive stimulation results in a varying division of excitation and a consequent separation of the three movements from one another, each becoming individually regulated. [Tritt die Zufuhr extero- und propriozeptorischer Erregung dazu, so erhält, wie wir schliessen dürfen, jede der drei Bewegungen ihre eigenen, voneinander verschiedenen Regulatoren, deren Tätigkeit eine verschiedene Erregungsverteilung bewirkt, durch die die Bewegungen voneinander getrennt werden." (p. 594)]. Von Holst emphasized that the fundamental rhythm itself is an *automatic* process in the spinal cord, independent of the periphery.

In support, von Holst cited the experiment of Adrian and Buytendijk (1931) (p. 45) on the isolated brain stem of the goldfish in which were recorded rhythmical potential changes whose frequency was the same as that of the normal respiratory movements (and, added von Holst, swimming and pectoral fin movements). He also referred to other investigations: Brown (1916) supposed that there was an automatic rhythmical basis for the locomotion of mammals, firstly because of the locomotory movements under narcosis in which he assumed the complete stopping of impulses from the periphery; secondly, because he was still able to observe locomotory movements after operative deafferentation. Winterstein (1908, 1911, 1913) came to a similar conclusion with regard to the respiration of mammals. Polimanti (1912c) found that in embryos of the dogfish (*Scyliorhinus*) the tail was already beating rhythmically before branchial respiration had begun and that after the onset of gill respiration both rhythms were synchronized for some time. Later this synchronization disappeared and von Holst suggested that at this time the connections with receptors were beginning to be established. Babak (1907, 1911) suggested a close relationship between respiratory and locomotory rhythms. In various fish embryos, before the onset of branchial respiration, he observed rhythmical fin and tail movements whose frequency was dependent upon the oxygen content of the water. In some fishes (cichlids) even the adult animal exhibits synchronized respiratory and pectoral fin movements. Von Holst explained all these observations on the basis of his theory. Equally (von Holst 1934c), he applied it to his studies of locomotory reflexes in *Anguilla* and *Misgurnus fossilis* (p. 59), concluding that all the various movements and reflexes can be regarded as modifications from the periphery, as a result of peripheral excitation, of a fundamental type of rhythmical movement coordination depending upon an automatic central process.

Von Holst (1935a) showed that the tench (*Tinca tinca*) can still swim after all the dorsal spinal nerves have been cut across excepting the two most anterior which innervate the pectoral fins (p. 67). While, on the one hand, this experimental result provided evidence against the chain reflex theory of locomotion, von Holst further maintained that it provided evidence in favor of the theory that the swimming rhythm is not a reflex at all but that the spinal cord itself generates automatic alternating impulses when it receives the necessary increased excitation. The releasing mechanism can be any one which will make the intact animal swim: tactile, static, optical, nociceptive, etc.

Von Holst (1934b, 1935a, 1936b, 1939) further elaborated his theory: In the anterior region of the spinal cord there are "automatic cells" in which there arises the automatic rhythmical process which is responsible for the rhythmical activity of the cord (e.g. rhythmical locomotory movements). These automatic cells continue to function as long as there is a certain level of the central excitatory state. This level is maintained by the medulla. Thus a medullary preparation shows continuous activity whereas a spinal preparation merely responds to reflex stimulation. The automatic cells can be separated into three functionally different regions with changing boundaries which may possibly be continuously labile, according to the activity of each of the three movements (respiratory, pectoral fins, and tail) and the incoming proprioceptive excitations. The automatic cells, in their turn, affect the motor cells which send impulses to the muscles. Their influence is not one of direct stimulation but, rather, an effect upon the balance of excitation of the motor cells when the latter are in a given state of activity as a result of peripheral or central influences of the most varied kinds. This effect consists of a rhythmical shift in a reciprocal sense of the balance of activity of the antagonistic groups of motor cells. The coordination of the segments of the spinal cord, as well as their rhythmical discharge, depends upon this purely central fundamental process and does not necessarily require incoming peripheral excitations. However, the central coordination is matched to the changes in external conditions through the peripheral receptors. The role of the latter must primarily be to vary the central coordination so that it fits the existing peripheral conditions.

A similar theory of an automatic mechanism within the central nervous system responsible for the control of rhythmical coordinated movements and not necessarily dependent upon any kind of afferent inflow has been put forward by Weiss (1941). It is based upon experiments on Amphibia and it may be referred to for the relevant arguments.

*e. The case for the dependence of locomotory activities upon peripheral stimulation.* Lissmann (1946a,b) considered that the various experiments in support of the theory of a central spontaneous automatic drive behind rhythmical locomotory movements were suggestive but by no means conclusive. Thus, no direct connection had apparently been shown to exist between the slow changes of electrical potential in the central nervous system which were alleged to represent the central drive and the impulses observed in motor nerves. Further, although it was maintained by supporters of the theory that, excepting in the case of some mammals, no alteration in the basic patterns of coordination followed extensive and even complete deafferentation, the fact had not been irrefutably demonstrated. He maintained that it was by no means certain "whether or not afferent inflow, however limited, constitutes an essential part of the mechanism which maintains locomotory rhythms, either as a pacemaker or by keeping the central excitatory state at an appropriate level" (Lissmann, 1946b, p. 162).

Lissmann argued that in most cases complete deafferentation of the body can produce no conclusive evidence, for a post-medullary spinal section will cut off afferent excitation from the cranial region and the animal, for that reason alone and without any additional deafferentation, will immediately cease to display a locomotory rhythm with any regularity. The dogfish, however, is an exception to this rule, since it carries out locomotory movements for long periods after post-medullary spinal section (p. 56). For this reason Lissmann made experiments on the spinal dogfish (*Scyliorhinus canicula* and *Squalus acanthias*).

He first studied the initial spreading of the undulatory posture in the animal recovering from anesthetic, in moribund preparations, and in animals whose swimming had been inhibited. The course of events is similar to that observed in the eel by von Holst (1935a) (p. 68) and Gray (1936a) (pp. 70, 76), i.e. two distinct processes are involved: first an undulatory posture is developed over the body and then this is propagated along the body. As the effect of the anesthetic becomes weaker, breathing movements appear. Later a strong pinch is followed by a single movement response of the body. Still later a pinch results in a movement response followed by a variable number of swimming beats, the number increasing as time goes on. Finally, in response to a stimulus or without any accountable reason, the persistent swimming rhythm emerges. Lissmann confirmed that the spinal dogfish, mounted by two pins passed through the snout, exhibits uninterrupted locomotory movements for some days. If, instead of being suspended in this way, the preparations were left in the tank, their behavior varied considerably, the fishes in many cases remaining motionless at the bottom. When any of these immobile specimens were held by the snout they invariably resumed their incessant swimming.

Lissmann pointed out that some of the discrepancies in the results of previous authors and his own observations could possibly be explained on the basis of some conclusions by Bauer (1926). This investigator described individual variations and differences arising from the different age of specimens or from the length of time which had elapsed since the operation or from reflex reversal after fatigue.

Lissmann considered von Holst's claim that the rhythmic-automatic neurones are quite distinct from the motor neurones and that the latter are excitable through reflex stimulation without the participation of the former. Lissman pointed out that it might then be expected that a reflex released in an actively swimming dogfish could be added as an independent response superimposed on the independent automatic rhythm. The swimming movements of the spinal dogfish are very regular and through an appropriate slight touch Lissmann was able to augment, accelerate, suppress, or reverse any single beat at any phase of the movement. When the rhythm emerged again with the next regular beat, the amplitude and frequency could be the same or nearly the same as before the application of the stimulus but the phase relation had changed in nearly every case. Lissmann pointed out that this resetting of the rhythm after a very short and feeble stimulation shows how susceptible the rhythmic elements are to outside influence. He concluded that "the response cannot be interpreted as resulting from a superposition of an independent automatic rhythm integrating with a reflex but must be considered as an interaction of two equal and mutually dependent processes" (p. 160). At the same time, the fact that a definite break in the response appeared whenever the rhythm emerged again after reflex modification may point to a certain degree of independence (Lissmann).

When a glass plate was gently placed against the ventral side of the animal in order to provide a diffuse touch stimulus, the swimming nearly always ceased, either almost immediately or after some delay. Lissmann concluded that this response was the result of inhibitory action and not of mechanical conditions from the observation that after careful removal of the glass plate the fish tended to remain inactive and might persist in this condition until a gentle tactile stimulus was applied. Once such a releasing stimulus had been applied the state of activity was maintained. Lissmann writes: "As the state of either activity or inactivity is maintained for prolonged periods under equal external conditions, and as that state is determined by the initial stimulus, it is hard to avoid the conclusion that in the case of activity the excitatory effect of the releasing stimulus sets up a sequence of re-exciting processes (such as proprioceptive reflexes or central self-exciting cycles) which are the further cause of the persistence of movement." (Lissmann, 1946a, p. 152).

The most obvious source of such a possible re-excitation could well be in the peripheral proprioceptors and Lissmann therefore investigated the responses of the spinal preparation after deafferentation. He carried out spinal section in the dogfish behind the medulla and cut across some or all of the dorsal roots excepting the last small ones at the end of the tail. These he isolated by a transverse section of the spinal cord. Lissmann referred to von Holst's earlier experiment on deafferentation in the tench (p. 78) in which, however, the nerves to the pectoral fins were left intact, observing that von Holst had also demonstrated the dominant role of these fins in influencing locomotory rhythms (p. 87).

After post-medullary section Lissmann found that exposure of the entire spinal cord did not affect the persistent locomotory rhythm of the spinal dogfish (Fig. 23, 1*a*). If anything, the activity was greater after exposure of the cord. After complete severance of all dorcal roots, either in stages or in one single operation, none of the fish ever showed the slightest spontaneous movement (Fig. 23, 1*b*). An additional cut through the spinal cord, either anteriorly or posteriorly, made the preparation twitch but never released a rhythmical response. Lissmann considered that this experimental result demonstrated the dependence of the persistent locomotory rhythm of a spinal dogfish upon afferent excitation. After partial deafferentation involving about half the number of the dorsal roots (either the anterior or the posterior half or unilateral) the rhythm was still present and practically indistinguishable from that shown by the spinal animal before deafferentation. Thus, as long as deafferentated musculature is connected through the spinal cord with normally innervated musculature it will take part in both tonic and rhythmic responses. After afferent excitation is cut off it ceases to do so (Fig. 23: 2, 3, 4). After extensive deafferentation exteroceptive stimulation of the preparation could sometimes still be followed by swimming movements but these did not persist. After complete deafferentation, excepting the tail extremity, the reaction of the dogfish to exteroceptive stimulation was never anything but a single response which usually threw the body into

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FIG. 23. The effect of deafferentation upon the locomotory rhythm of the spinal dogfish. The white regions represent the desensitized areas of the body. The level of spinal transection is indicated by a thick line. ∿∿∿ = rhythm; —— = inactivity. 1*a*: Persistent rhythm of a spinal *Scyliorhinus canicula*. 1*b*: The rhythm is abolished after deafferentation. 2*a*: Persistent rhythm of an anteriorly deafferentated *Scyliorhinus*. 2*b*: Disappearance of the rhythm after complete deafferentation except for the tail. 3*a*: Rhythm after deafferentation of the posterior body region. 3*b*: After transection of the cord at the level of deafferentation the deafferentated zone becomes silent. 4*a*: Persistent rhythm of a spinal *Squalus acanthias*. 4*b*: The rhythm does not appear modified after complete unilateral deafferentation. 4*c*: Bilateral deafferentation abolishes the rhythm (from Lissmann, 1946b).

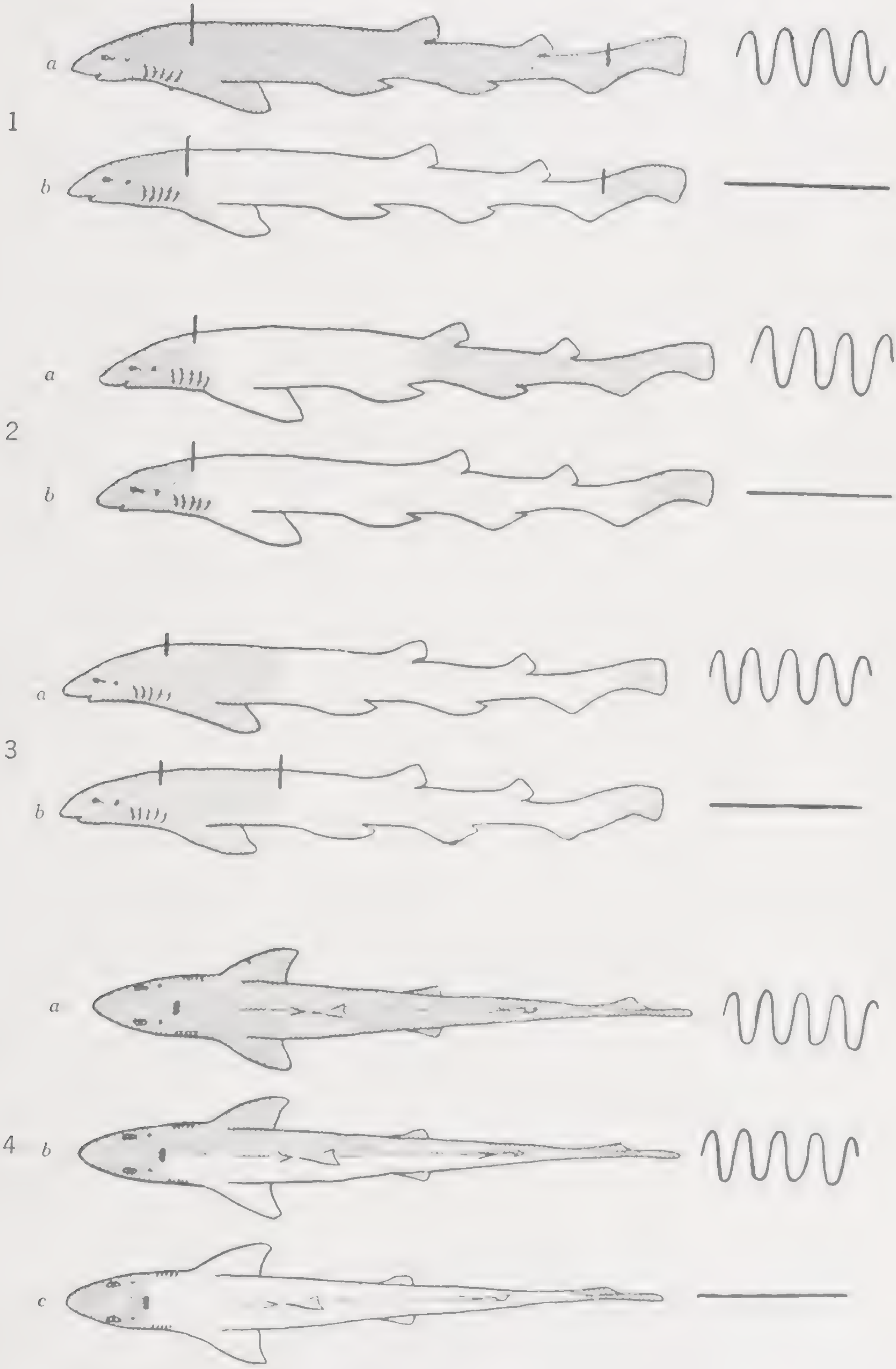

FIGURE 23

an S-shape. No rhythmic response was ever obtained. In one instance a normal dogfish (*S. canicula*) (i.e. not spinal) was completely deafferentated excepting for the tail. After the operation there were no more swimming movements, either spontaneously or after stimulation.

Lissmann also made a preliminary study of the effects of electrical stimulation applied to the cord of the spinal dogfish. The spinal cord was either lifted onto two steel electrodes or the electrodes were placed on each side of the cord. Shocks were applied from an induction coil, either singly or by an interruptor.

In one experiment the electrodes were inserted just posterior to the second dorsal fin in an anesthetised animal. During recovery from the anesthetic, at a stage before any reflexes could be elicited, a short burst of shocks (ca. 0.5 second) was followed by a rhythmical response of three beats of large amplitude and low frequency, both decreasing towards the end of the response. As recovery proceeded a similar burst resulted in an increasing number of beats and the decrease of amplitude and frequency was less rapid. Finally the stimulation was followed by the persistent swimming movements. From then onwards the effect of stimulation was a temporary increase in the amplitude and frequency of these movements.

In other experiments Lissman applied stimulation from an induction coil to the cord of the spinal dogfish, either swimming actively or inhibited through ventral touch (p. 81). Continuous weak stimulation was usually followed by an asymmetrical tonic contraction on which were superimposed very rapid beats of small amplitude whose frequency did not appear to have any relationship with the frequency of stimulation. On increasing the strength of stimulus there appeared a rhythmical response of wide amplitude and low frequency. At intermediate strengths of stimulus these two responses were superimposed on one another. Stimulation applied to the spinal cord of an extensively deafferentated dogfish produced a rhythmical response much more readily than did exteroceptive stimulation. In two cases the response persisted for a short time after discontinuing the electrical stimulation. In both these cases, apart from the tail, one or a few pairs of dorsal roots had been left intact in the region of the dorsal fins. When only the tail was intact the rhythm did not persist after stimulation was stopped. Two completely deafferentated animals whose spinal cords were stimulated electrically responded with only a single contraction. There was no rhythmical response.

Lissmann criticized the interpretation of swimming movements on the basis of an essentially central mechanism, finding that its complications did not always appear plausible. "It is difficult to visualize the reasons why the medulla, the rhythmic activities of which in the isolated state have been related to the swimming rhythm, should exert a *steady and continuous* effect on other elements in the spinal cord which are termed the 'automatic-rhythmic cells,' and which in turn are said to affect rhythmically the 'motor-cells' (von Holst, 1934a,b,c; 1935a,b,c; 1936a,b,c; 1939). No conclusive evidence appears to have been produced to show the existence of an independent automatic-rhythmic process onto which reflex responses may be superimposed through direct excitation of the motor cells. As long as the animal is at rest neither the strength of any locomotory reflex nor the reflex time have been demonstrated to alter rhythmically in such a way that it might appear reasonable to ascribe such changes to a concealed, continuous, inherent rhythm in the central nervous system playing a 'predetermined score' (Weiss, 1941)." (Lissmann, 1946a, p. 159).

Lissmann concluded, on the other hand, that the results of his deafferentation and stimulation experiments suggested that the maintenance of the rhythmical locomotory movements in the spinal dogfish depends upon re-excitation of the system and that afferent stimuli from the periphery play an essential part in this process of re-excitation as well as in the process of release. He considered that the appearance of the rhythm after exposure of the spinal cord and dorsal roots and partial severing of the latter made it unlikely that the shock of the operation could account for the results obtained from fully deafferentated preparations.

Lissmann observed that while it is not difficult to visualize afferent impulses entering the spinal cord after the preparation has started swimming, the cause of the initial stroke in the spinal dogfish has not been determined. He pointed out (p. 173) that continuous discharges have been recorded from nerves connected to unstimulated sense organs and from skate and dogfish stretch receptors under constant tension and suggested that the releasing stimulus for movement may be such "spontaneous discharges" in the sensory nerves or stimuli arising out of the environment.

Lissmann concluded that locomotory activities, as shown by the spinal dogfish preparation, are not the result of an automatic central drive and central pattern of activity but rather that they are of a reflex nature, being "moving sites of nervous integration continuously re-excited from within or without the central nervous system and ruled by coordinating patterns of afferent impulses."

For various points and arguments drawn from other vertebrate classes which bear on the problem of the nervous control of locomotion in its wider aspects reference may be made to the papers by Weiss (1941), Beritoff (1948), ten Cate (1949a), and Gray (1950).

## 6. The Coordination of Fin Movements

Some fishes swim largely by means of extensive body movements while others keep their bodies relatively still and swim mostly with their fins. Von Holst (1935b,c; 1936a,b,c) investigated the relations which exist between the movements of the various fins in the latter type of swimming. He found that in *Labrus festivus* the pectoral fins and the hinder enlarged portion of the dorsal fin are those chiefly used, although occasionally the anal and tail fins are used too. The pectoral fins mostly move alternately but now and then they move in the same direction together. The dorsal fin has a quicker rhythm of movement than the pectoral fins and it moves with great regularity as long as it alone is active. However, if the pectoral fins start to beat, changes are seen in the rhythm of the dorsal fin.

Von Holst cut through the medulla of *Labrus* posterior to the exit of Nerve III and anterior to the exit of Nerve X and so prevented the brain from influencing the activity of the spinal cord. After this operation the respiratory movements stop and the fish must be provided with a stream of water over its gills. The spinal cord becomes excitable in 2–10 hours and rhythmical fin movements finally appear without apparent stimulation. These continue for as long as 6 days and may be recorded on a kymograph. Generally they are very regular but in some individuals there may be slight pauses, either of all the fins together or of the dorsal fin alone or of one or both pectoral fins alone.

With such a preparation von Holst found that the frequency and amplitude of the pectoral fin rhythm remain regular whether the dorsal fin rhythm is active or not. The dorsal fin rhythm, on the contrary, is only regular if both pectoral fins are motionless. As soon as they move the dorsal fin shows pronounced variations in its beating. Fig. 24 shows a record of the fin movements of a *Labrus* preparation. In *a*, about 40 minutes after the operation, the dorsal fin is beating evenly and both pectoral fins are motionless. In *b*, about 9 minutes later, one pectoral fin has begun to move and the effect upon the dorsal fin is clearly visible. In *c* the second pectoral fin is also beating and the effect upon the dorsal fin is still more pronounced.

Von Holst showed that this effect upon the movements of the dorsal fin is not caused by currents of water generated by the movements of the pectoral fins and concluded that it must be the result of some central nervous process. Since analysis of the records led von Holst to believe that some influence associated with the pectoral fin rhythm directly affects the dorsal fin rhythm, he suggested two possible mechanisms: either the movements of the pectoral fins produce excitations initiating a reflex which influences the dorsal fin rhythm or else the central process itself, which he considers is responsible for the pectoral fin rhythm, has some effect upon the central process which controls the dorsal fin rhythm. Von Holst decided the question by experiment. As already stated, the pectoral fins of the preparation occasionally stop beating. By attaching them to a suitable system of rods during these still periods von Holst was able to move them in a reasonably natural manner. This must have stimulated proprioceptors and, if the first possibility had been correct, must accordingly have influenced the dorsal fin rhythm. Since it did not do so von Holst concluded that the effect was produced by some central nervous mechanism.

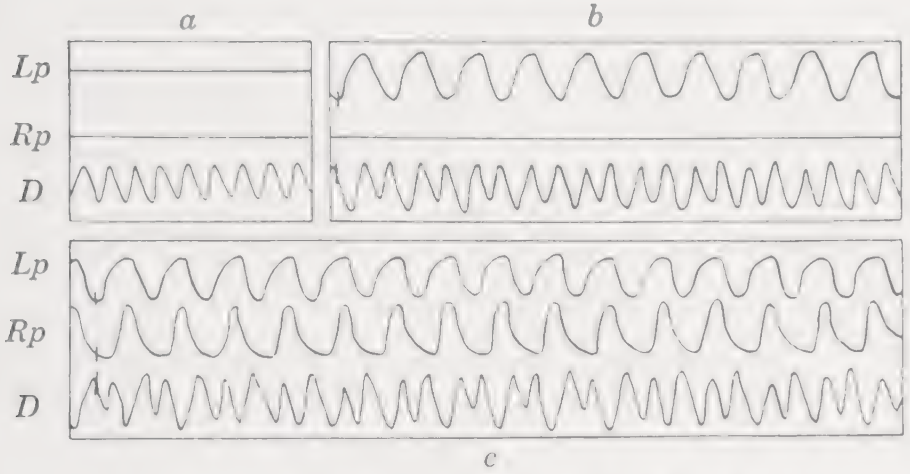

FIG. 24. *Labrus*: records of the movements of the left and right pectoral fins (*Lp*, *Rp*) and of the dorsal fin (*D*). In *a* only the dorsal fin is active, in *b* the left pectoral fin is also moving; in *c* all three fins are active. The pectoral fins beat alternately: the rising side of the tracing corresponds to a beat backward in the case of the right pectoral fin and to a beat forward in the case of the left (from von Holst, 1936a).

Von Holst proposed the term "*relative coordination*" for this type of movement relationship between the fins in distinction to the "*absolute coordination*" which is found in higher vertebrates, particularly in association with life on a solid substratum, and in which there is always a definite relation between the phases of movement of the different limbs. Since the pectoral fin rhythm in *Labrus* appeared to influence that of the dorsal fin but the reverse effect was not observed, von Holst referred to the former as dominant (dominierend) and the latter as dependent (abhängig). The same relationship was found in *Sargus*. Further, he found that in *Labrus* the tail fin rhythm is dominant over the dorsal fin rhythm and that in *Sargus* the pectoral fin rhythm is dominant over the tail fin rhythm. He also observed relative coordination between the two pectoral fins, i.e. between two rhythms which are of equal value. Here the dependence is usually mutual although it can happen that one fin is dominant.

*The phenomenon of "block."* Von Holst found that in *Sargus* and *Labrus* the two pectoral fins usually beat alternately at the same frequency, i.e. while one moves forwards the other moves backwards. These movements of the right and left pectoral fins are equal; they are not dependent upon any other dominant rhythm and a mutual dominating influence will not become apparent under these conditions. However, one pectoral fin may stop moving for a time. When this occurs it takes place suddenly and without warning. Similarly, when movement starts again, it does so suddenly and completely. The duration of each pause is exactly equal to that of one rhythmical beat of the fin (or, seldom, to a whole number of rhythmical beats). While such a stopping of the pectoral fin beat on one side can happen periodically, e.g. at each 4th (Fig. 25), 3rd, or 2nd beat, the pectoral fin on the other side may go on beating regularly (not shown in the figure). Von Holst referred to this periodic stopping as "*block*" because of its superficial similarity to the phenomenon of block in the heart and considered it to be the result of the stopping of central automatic processes and not, as in the heart, of an interruption of the conduction from one rhythmically active part to another.

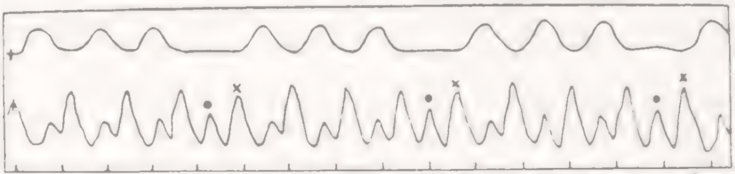

FIG. 25. *Labrus:* upper trace = left pectoral fin; lower trace = dorsal fin. Time in seconds (from von Holst, 1935c).

The lower curve in Fig. 25 shows the dependent rhythm of the dorsal fin. In the example shown here there is a relation of 1:2 between the frequencies of the pectoral and dorsal fin movements so that the strength of the dominant influence can be recognized in the difference in height of the two neighboring peaks of the curve. Inspection of the two dorsal fin beats which occur during the pause of a pectoral fin beat shows that each time the little peak (marked ●) is raised while the bigger (X) is lowered, i.e. the inhibiting and the strengthening effects are both reduced by a certain amount. The amount by which the effect of the dominant rhythm is reduced is equivalent to the stopping of movement of the one pectoral fin and von Holst concluded that the block phenomenon appears to depend on the periodic stopping of the one automatic central rhythmical process.

It has been suggested several times that the central rhythms may be brought about by the production, through the metabolism of special cells, of an exciting substance. As soon as a critical concentration of this substance is reached it provides the stimulus for the motor ganglion cells of the appropriate muscles. The discharge of the motor cells destroys the exciting substance and the process starts again. If this stimulating substance spreads into the region of another rhythm with its own frequency of production, there will be changes in the momentary concentration of the exciting substance there. These varying concentrations result in the stimulation of correspondingly varying numbers of motor units, i.e. in alterations in speed and duration of contractions (see p. 77).

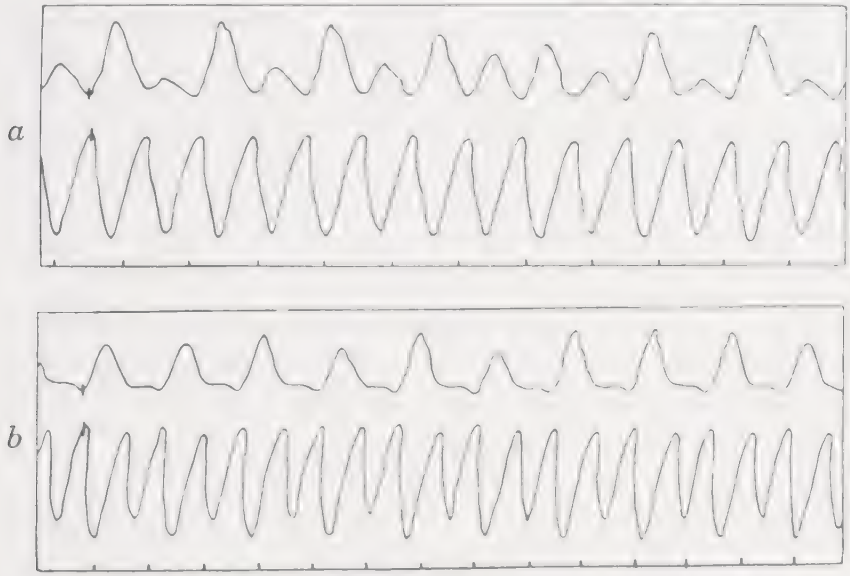

Fig. 26. *Sargus:* upper trace = right pectoral fin; lower trace = tail fin. Record *b* was made shortly after record *a* from the same animal. Time in seconds (from von Holst, 1935c).

On the basis of the above conception von Holst believed that the phenomenon of block is the result of a deficiency in the automatic formation of the stimulating substance which is concerned with the movements of one pectoral fin.

*The phenomenon of "alternans."* Von Holst also observed the appearance of regular alternate strong and weak beats in association with the movements of the pectoral, dorsal, and tail fins and referred to this phenomenon as "*alternans.*" Typically this phenomenon is seen as a beat larger than normal alternating with one smaller than normal so that the sum of two consecutive beats is equal to that of two normal beats, as shown in Fig. 26*a*, *b*. If $n$ is the number of contracting muscle fibers in a normal case, the alternans is the result of $(n + x)$ and $(n - x)$, in which $x$ represents a varying number. In the example shown in Fig. 26 *a* the value of $x$ becomes less towards the middle of the record, becoming greater again subsequently (Fig. 26*b*). In this example there is an alternans of one pectoral fin of *Sargus* while the other (not shown) goes on beating regularly. The lower record shows that the tail rhythm is strongly dependent upon the dominant pectoral fin rhythm.

In general von Holst considered that these central rhythms bear many similarities to those shown by the heart. Thus, in addition to block and alternans he described the appearance of extra rhythms which appear out of turn and are followed by a normal period without a compensatory pause. Their amplitude is smaller the shorter the time between the extra rhythm and the previous regular movement. Suitable stimulation, such as touching or pressing different parts of the body, leads to increase or inhibition of the activity of the spinal cord, recalling the effects of acceleration fibers and vagus stimulation on the heart. He also concluded that, under any given conditions, there is either a definite action of constant intensity (maximal) or else no action at all, i.e. that the all-or-none principle may be applied to this type of activity in the spinal cord.

The phenomenon of relative coordination, according to von Holst, depends upon the presence of two or more automatic rhythmical stimulus-forming processes of which one (the dominant) produces in the other (the dependent) alterations in frequency and speed of movement which follow definite rules. These alterations come about through superposition of the one rhythmical automatism upon the other, this being a central process. Von Holst also described a "*magnet effect*"—the tendency for the frequency of one rhythm to attract the frequency of another. Superpositioning may be in the same or the opposite sense, the central processes being accordingly strengthened or weakened. (Or, in terms of the rhythms as shown by the fins, the effect of one fin movement upon another depends upon their phase relations so that all degrees of addition and subtraction may take place.) This central superposition can only be concerned with processes which are of the same nature. Thus excitation and inhibition of rhythms as a result of peripheral stimulation are not of this nature (von Holst).

Von Holst (1939, 1948) has further discussed these observations and has examined the theoretically possible effects which may arise through the interaction of two and of three rhythms.

## VII. THE AUTONOMIC NERVOUS SYSTEM

### A. Anatomy

The anatomy of the autonomic nervous system has been little investigated in cyclostomes. The sympathetic ganglia do not appear to form a chain, excepting possibly in the head, but a diffuse system which is generally represented by scattered cells whose connections with other parts of the nervous system are unknown. The vagus supplies both the gut and the heart.

In the elasmobranchs the sympathetic ganglia form an irregular series lying along the body and not extending into the head. Pre-ganglionic fibers pass to them from the spinal cord and post-ganglionic fibers passing from them end in the smooth muscles of the gut and arterial walls. They do not send fibers to the spinal nerves by way of recurrent grey rami and thus do not supply the skin. There is a well-developed vagus innervating the heart and gut (Young, 1933a). Nicol (1950) has investigated the relations of the autonomic system in Holocephali (*Hydrolagus*).

In teleosts the sympathetic ganglia form a chain which extends along the body as far forwards as the trigeminal nerve. The pre-ganglionic fibers for the head region arise from the spinal cord in the trunk region and pass forward along the chain to the cranial sympathetic ganglia. Unlike the condition in elasmobranchs, post-ganglionic fibers pass to the skin by way of the recurrent grey rami. Little is known about the parasympathetic system. Young (1930) has shown that fibers of this system pass in Nerve III to the iris to work against the sympathetic fibers. The vagal system is well developed but no parasympathetic fibers have so far been found in other cranial nerves or in association with a sacral system. The classical pattern of Langley (1921), based largely upon mammalian studies, does not apply to the teleosts. In these fishes "it is not possible to divide up the autonomic nervous system into sympathetic and parasympathetic divisions by either anatomical, physiological, or pharmacological criteria. Presumably the two 'antagonistic' systems found in mammals are a late development, allowing for a delicate balancing of activities for the maintenance of homeostasis" (Young, 1950, p. 214).

For accounts of the autonomic nervous system of fishes reference may be made to the papers by Young and by Nicol listed in the bibliography. The subject as a whole has recently been well reviewed by Nicol (1952). The account which follows will therefore be limited to the main conclusions in his article apart from a few topics which have arisen since it was written or, for other reasons, were not included in it.

### B. Functions Associated with the Alimentary Canal (and see Chap. III, Vol. I)

In the cyclostomes electrical stimulation of the vagus causes contraction of the sphincter of smooth muscle which lies between the pharynx and the intestine. Authors vary in their reports of the effects of electrical stimulation of the vagus upon intestinal tone.

In elasmobranchs the vagi contain motor fibers running to the esophagus and stomach. Electrical stimulation of the visceral branch of the vagus results in local contractions and peristalsis of all regions of the stomach. There is no definite physiological evidence for vagal innervation of the intestine. Sympathetic fibers provide a motor innervation for the stomach and intestine. The digestive glands do not appear to receive any autonomic fibers for the control of secretion.

In the teleosts the stomach receives a motor supply from the vagus and contracts when the latter is stimulated electrically. The vagus appears to be without effect upon the intestine. In some species the splanchnic nerves provide a motor innervation for the stomach, their stimulation being followed by contraction. In all the species which have been examined electrical stimulation of the splanchnic nerves is followed by movements of the intestine, pyloric caeca, and rectum. The action of autonomic drugs is inconsistent and often cannot be reconciled with the results of electrical stimulation. Little is known of the nervous control of digestive secretion in teleosts. (For further details see Nicol, 1952.)

### C. Functions Associated with the Swim-Bladder (and see Chap. IV)

Bohr (1894) showed that the secretion of gas in the swim-bladder is under nervous control. He found that section of the vagus in the cod (*Gadus callarias*) and the perch (*Perca fluviatilis*) is followed by either no change or a slight decrease in oxygen content of the swim-bladder. If, however, the swim-bladder is empty, it remains in this condition. Transection of the sympathetic nerves results in a rise in the oxygen content of the bladder in the cod. This last effect was considered by von Ledebur (1937) to be explained on the basis of an oxygen-secretory function associated with vagal activity and an absorptive or inhibitory function associated with the action of the sympathetic system.

Fänge (1953), working on a number of teleosts, showed that the tonus of the muscles associated with the secretion and reabsorption of gas is controlled by the intestinal branch of the vagus. Section of the sympathetic nerves to the swim-bladder does not appear to affect the tonus of the mucosa. The slight rise in oxygen content which follows this operation was considered by Fänge to be probably the result of extinguishing vasoconstrictor impulses to the gas gland. From the results of experiments involving electrical stimulation and the presentation of autonomic drugs Fänge concluded that the intestinal branches of the vagus may well contain adrenergic as well as cholinergic fibers. Such a mixture of antagonistic fibers might account for the apparently anomalous effects which follow the administration of autonomic drugs and which have more than once been observed in connection with other organs in teleosts receiving an autonomic innervation (see Young, 1936).

### D. Functions Associated with the Urinogenital System

In the elasmobranchs the urinogenital organs receive no parasympathetic innervation but stimulation of sympathetic fibers is followed by contraction of the oviducts.

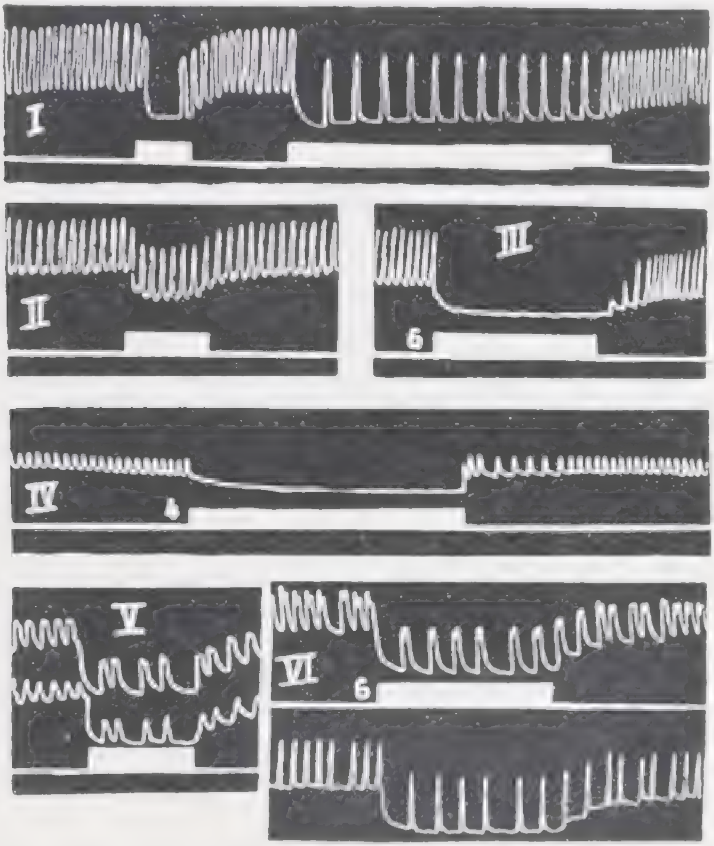

FIG. 27. Action of the vagus on the heart of *Scorpaena*. I. entire heart; II. weak excitation causing negative tonotropic effect, III. auricle alone, IV. ventricle alone, V. double record showing powerful negative tonotropic effect. VI. double record showing positive inotropic and negative tonotropic effects (from Jullien and Ripplinger, 1951a).

The few experiments which have been carried out on teleosts in this connection indicate that stimulation of the sympathetic chain is again followed by movements of the ovaries. The effects of autonomic drugs are inconsistent. (For further details see Nicol, 1952.)

### E. Functions Associated with the Heart and Vascular System (and see Chap. II, Vol. I)

In the cyclostomes electrical stimulation of the vagus is reported not to have any effect upon the hearts of *Myxine* and *Polistotrema*. Cardioregulatory fibers have, however, been described in the vagus of the lamprey (*Petromyzon*).

In the elasmobranchs the heart appears to receive no accelerating fibers but it receives inhibiting fibers through the vagus, the stimulation of which slows or stops its beat. The arterial blood pressure is probably under nervous control although direct evidence for this has not been produced.

In teleosts cardiac augmentor nerves have been said to be absent. The vagus has been reported as having a powerful inhibitory action on the sinus venosus and the auricle but no effect on the ventricle.

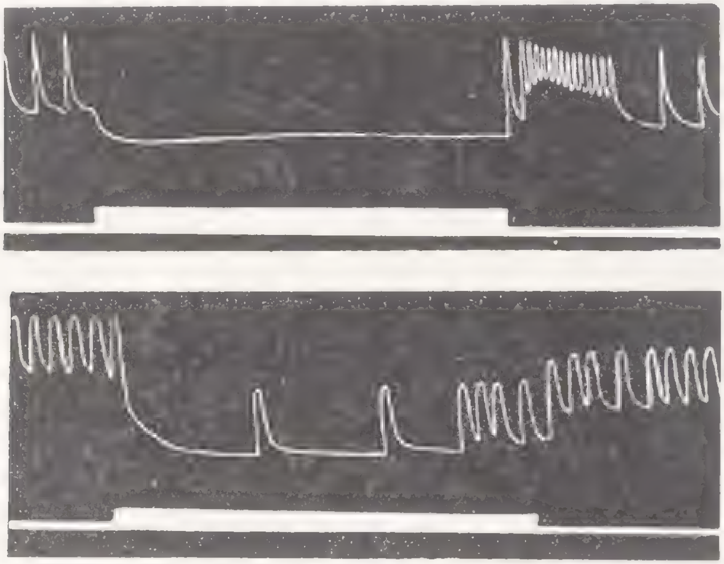

FIG. 28. Action of the vagus on the heart of *Belone* (above) and *Conger* (below) (from Jullien and Ripplinger, 1951b).

Jullien and Ripplinger (1950a,b,c) investigated the innervation of the heart in the tench (*Tinca*). They found that on electrical stimulation of the vagus the beats became slower (negative chronotropic effect) and finally stopped excepting for an occasional irregular contraction. Further, they noted that the heart stopped in diastole with a much lower tone than is usual in the relaxed condition (negative tonotropic effect). If, instead of stimulating the vagus, acetylcholine was applied to the exposed heart, the latter began to beat arhythmically or, if the concentration of acetylcholine was high enough, it stopped altogether. However, after this treatment with acetylcholine, the tone was only very slightly below that associated with normal diastole. If, when such a heart was either beating slowly or had stopped, the vagus was stimulated electrically, the tone fell to an abnormal extent and the heart stopped beating, if it had not already done so through the action of the acetylcholine. On discontinuing the electrical stimulation of the vagus the tone rose again to the value which it had had before the electrical stimulation was applied. Jullien and Ripplinger argued that the decrease in tone which followed electrical stimulation of the vagus could not be the result of acetylcholine formation, since much greater amounts of acetylcholine applied directly did not have this effect. They therefore believed that it was the result of the action of specific noncholinergic and purely negatively tonotropic fibers.

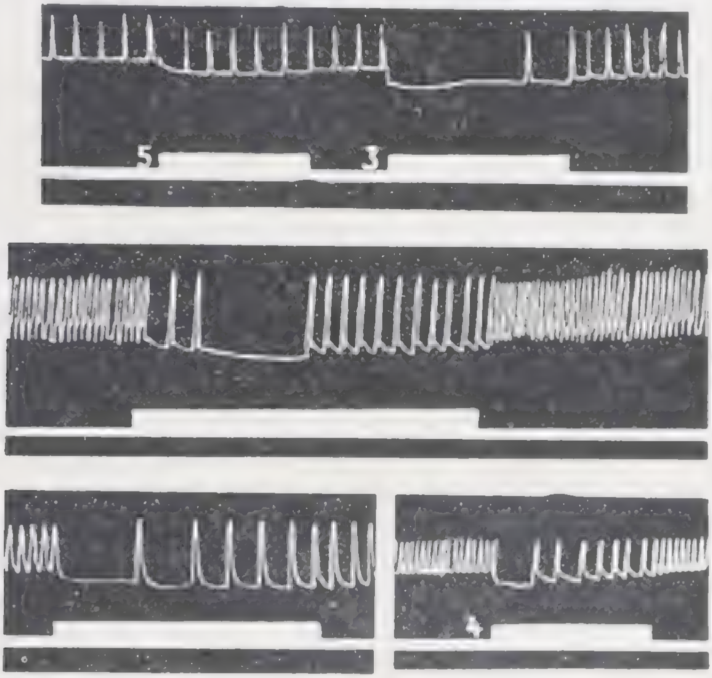

FIG. 29. Action of the vagus on the heart of *Mustelus* (top), *Sargus* (middle), the ventricle of *Sargus* (bottom left), and the entire heart of *Crenilabrus* (bottom right) (from Jullien and Ripplinger, 1951b).

In other papers (Jullien and Ripplinger 1950d; 1951a,b,c,d,f; 1952a,b; Ripplinger, 1952) they carried out similar experiments on the hearts of the elasmobranch *Mustelus* and the teleosts *Sargus*, *Crenilabrus*, *Scorpaena*, *Belone*, *Conger*, and *Esox*, the last two species giving the best reactions. They made the following generalizations (see Figs. 27–30):

(1) The threshold for the negative tonotropic effect is lower than that for the negative chronotropic effect and it is therefore possible, by using stimulation of low intensity, to dissociate the two and to obtain the negative tonotropic effect alone without changing the rate of heart beat. The delay in obtaining the negative tonotropic effect on stimulation of the vagus is short compared with the delay in the appearance of the negative

chronotropic effect.

(2) The negative chronotropic effect of vagal stimulation is seen in all parts of the heart but the auricle is the most sensitive. At a given intensity of stimulation it is possible to stop the auricle while the sinus continues to beat with a slower rhythm, initiating the ventricular contractions through the immobile auricle.

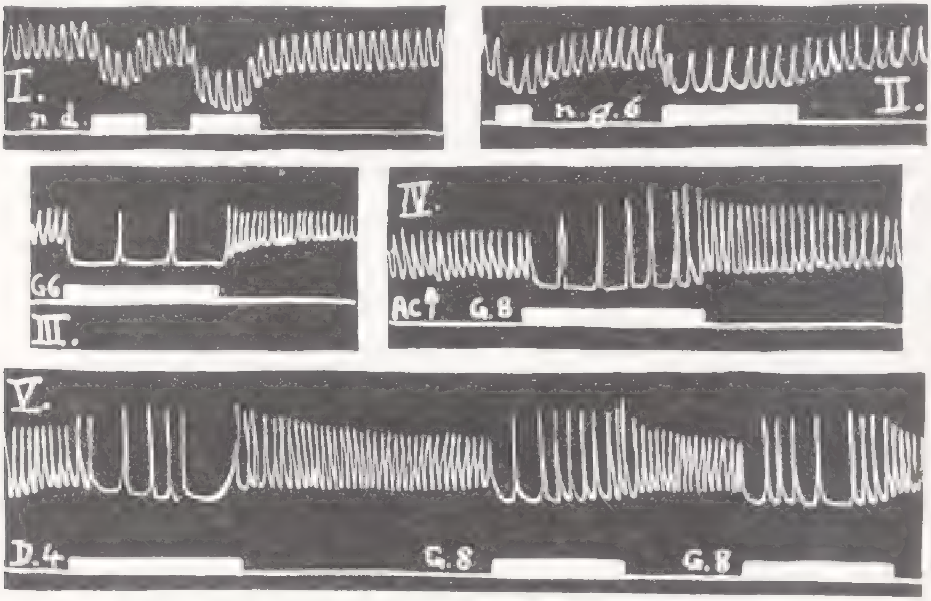

FIG. 30. Effects of vagal stimulation upon the heart of the pike, *Esox*. I. Excitation of the right cardiac branch of the vagus produces only a negative tonotropic effect; II. and III. Excitation of the left nerve in the same individual produces relaxation of the heart together with a negative chronotropic effect which is weak in II and very marked in III combined with stopping of auricular contractions. IV. The external application of acetylcholine to the heart of another individual is without effect. Subsequent excitation of the vagus is effective. V. The two branches of the vagus are negatively chronotropic and tonotropic; stimulation results in strengthening of the ventricular contractions initiated by the pacemaker while the auricles are stopped (from Jullien and Ripplinger, 1951f).

(3) Similarly the negative tonotropic effect involves the whole heart but is most marked in the auricle.

(4) The extent to which the tone of the heart is lowered by vagal stimulation varies greatly among different species.

Jullien and Ripplinger (1951e) also compared the right and left vagal branches with regard to tonotropic and chronotropic effects. In the roach (*Rutilus*) the left vagus contains only (or mostly) negative tonotropic fibers while the right appears to contain both types. In the tench (*Tinca*) the left vagus is chronotropic while the right is mixed. Further, the distribution of fibers varies from individual to individual.

They also estimated the amounts of acetylcholine released by vagal stimulation in hearts which had been previously treated with eserine. In the tench (*Tinca*) and carp (*Cyprinus*) the amounts produced were greater than in the pike (*Esox*). They failed to detect any in the roach (*Rutilus*). (Jullien and Ripplinger, 1950f,g).

Autonomic drugs were once again found to give apparently inconsistent results. Atropine suppresses the vagal chronotropic effect but not the tonotropic. Acetylcholine–atropine antagonism is very incomplete. Eserine makes the heart beat more slowly but never produces a fall in tone. Nicotine does not affect the chronotropic fibers but reverses the tonotropic action. In general, the auricle is more sensitive to drugs than the ventricle and the two parts of the heart often show opposite effects (Jullien and Ripplinger, 1950e; Ripplinger, 1950, 1951). (For further details see Nicol, 1952.)

### F. Functions Associated with the Iris

In elasmobranchs the contraction of the iris sphincter is not under nervous control but occurs as a direct response to illumination. On the other hand, the radial muscles of the sphincter are innervated by motor fibers from the ciliary nerves (Young, 1933b).

In teleosts it was shown for *Uranoscopus* by Young (1931, 1933c) that the sympathetic system brings about active constriction of the pupil while the parasympathetic controls its dilation. Stimulation of the sympathetic supply to the eye also results in retraction of the eyeball. The action of autonomic drugs appears to be inconsistent. (For further details see Nicol, 1952.)

### G. Functions Associated with the Control of Chromatophores (see also Chap. VIII)

Ballowitz (1893) demonstrated histologically that the melanophores of a number of teleosts are innervated by fine branches derived from more than one neurone. Similar observations were made by Wyman (1924) on the melanophores of *Fundulus heteroclitus* and by Whitear (1952) on those of the minnow (*Phoxinus*) (see Gray, 1955b). It was shown experimentally by Pouchet (1876) that color changes in various flat fishes are under the control of the sympathetic nervous system. Von Frisch (1911a) investigated the course of the sympathetic fibers of the minnow (*Phoxinus*). He cut the sympathetic chain and the spinal cord at different levels and severed spinal and cranial nerves, following these operations by observations on the dead fish and by studying the reactions of the living fish to black and white backgrounds and to electrical stimulation. Von Frisch showed that fibers causing aggregation of the melanophores pass from the medulla along the spinal cord, leaving the latter at about the level of the 15th vertebra to enter the sympathetic chain. There the fibers (or their post-ganglionic associates) run forwards and backwards, passing out with the spinal nerves to supply the melanophores on the surface of the body and with a branch of the trigeminal nerve to supply those on the head (Fig. 31). Electrical stimulation of these chromatic fibers results in paling of the region distal to the point of application of the stimulus (i.e. distal with regard to the distribution of these fibers). Section of the path of the chromatic fibers is followed by darkening of the distal region. Von Frisch further found that about half an hour after death the minnow, at first dark, suddenly becomes fully pale. He traced this paling to some activity of the spinal cord and believed that this contained a paling center from which impulses passed out to produce aggregation of the melanophores. However, he pointed out that the dying nerve fibers in the spinal cord might be undergoing processes which give rise to excitation (see Healey, 1954). Further experiments indicated the presence of another paling center at the anterior end of the medulla and the possible presence of a darkening center, inhibiting the paling center, in an unspecified anterior region of the brain.

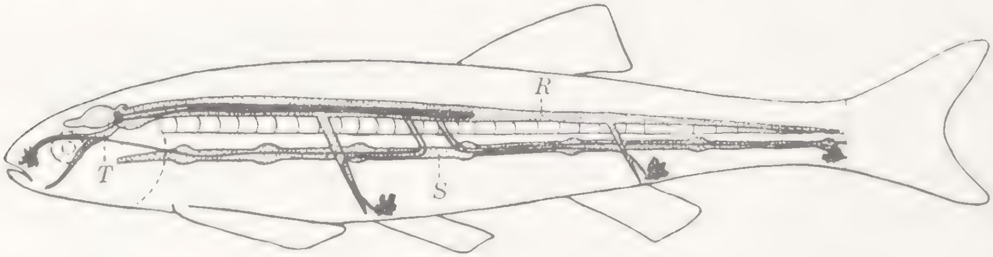

FIG. 31. Paths of aggregating chromatic fibers in the minnow, *Phoxinus*. R = spinal cord; S = sympathetic chain; T = trigeminus (from von Frisch, 1911a).

Von Frisch concluded that the melanophores are held in a state of tonic aggregation by the paling center in the medulla and that the darkening of the minnow on an illuminated black background is the result of an inhibiting action of the anterior brain center upon the aggregating center so that the melanophores assume their resting condition, i.e. become dispersed. He attributed the distal darkening which follows chromatic nerve section to disconnection of the melanophores from the tonic aggregating center, i.e. to their paralysis.

Schaefer (1921), working on *Pleuronectes*, failed to obtain darkening on stimulation of any region of the brain but, in all other respects, confirmed von Frisch's observations on the minnow. His general conclusions have been confirmed for teleosts by a number of workers.

Bert (1875), without providing any supporting evidence, first suggested that the melanophores in the chameleon receive a double innervation of sympathetic and parasympathetic fibers for active melanophore aggregation and dispersion respectively. Von Frisch himself (1911a) was aware of this possibility in the minnow but dismissed it in the absence of any evidence at that time. Since then the literature about double innervation of chromatophores has become extensive; it is readily accessible and the subject is treated here only in outline. The evidence is, generally speaking, indirect. There are many recorded observations which can be explained by assuming double innervation but which do not in themselves prove its presence (Healey, 1951). Added to this, the subject has been so repetitively discussed that there has been a strong tendency to confuse opinion with dogma.

Spaeth and Barbour (1917) treated isolated skin from *Fundulus* with ergot drugs and found that the melanophores first became aggregated and then moderately dispersed. The addition of adrenaline, which normally causes aggregation of the melanophores (Bray, 1918), now resulted in their maximal dispersion. One possible explanation was advanced that the motor sympathetic endings had been paralyzed by ergot and the adrenaline was now stimulating dispersing fibers. Vialli (1927) and Ciabatti (1929) reported similar reversal of the effect of adrenaline after preliminary treatment with ergotine, yohimbine, and other drugs in *Gambusia* and *Scardinius*. Giersberg (1931) treated minnows (*Phoxinus*) with ergotamine and choline and then stimulated electrically. He found that black flecks appeared temporarily and concluded that some of the melanophores were supplied with an actively dispersing innervation. Von Gelei (1942) attempted to trace the path of dispersing fibers in the minnow. After injecting ergotamine and acetylcholine he cut the sympathetic chain and the spinal cord at different levels and then applied electrical stimulation to the medulla. He concluded that there are dispersing fibers and that they do not follow the same path as von Frisch's aggregating fibers but pass from the spinal cord to the sympathetic chain with the first or second spinal nerve. There they run backwards and pass to the melanophores. That they do this by way of the spinal nerves is assumed, although no evidence for this is given. Section of the sympathetic chain in the anterior region of the normal minnow (as already shown by von Frisch, 1911a) does not appear to affect the rapid color changes of the posterior part of the animal. These dispersing fibers of von Gelei, if they do in fact exist, seem to be much less effective than the opinions of Parker (1948) would suggest. (See Healey, 1954; Gray, 1956b.)

Ergotamine has long been known to have a reversal effect on adrenaline

(Dale, 1906, 1913). In mammals it does not paralyze the sympathetic system but renders the end-organs incapable of reacting normally to the adrenaline released from the sympathetic nerve endings (see e.g. Goodman and Gilman, 1955). Gray (1955a), in criticizing the experiments of Giersberg and of von Gelei, has suggested that ergotamine may cause the melanophores to disperse instead of aggregate in response to adrenaline produced from the ends of melanophore-aggregating fibers as a result of electrical stimulation. In such an interpretation there is no need to postulate the presence of dispersing fibers.

Parker (1934, 1941, 1948) claimed to have provided evidence for double innervation by experiments made by himself and his collaborators on the killifish (*Fundulus heteroclitus*) and the catfish (*Ameiurus nebulosus*). This evidence is derived almost entirely from the study of caudal bands—small denervated regions in the tail—and is fully presented in his monograph. He maintained that the distal darkening which follows section of chromatic nerve fibers is the result of the mechanical stimulation of cutting whereas electrical stimulation has the greater effect upon the aggregating fibers. A cold block results in a temporary fading of the dark band distal to the block, indicating that continuous impulses are passing from the cut surface. Such an interpretation of the contrasting effects of cutting and stimulating chromatic fibers has met with considerable criticism (Sand, 1935; Waring, 1942; Young, 1950) and can not be accepted without much more evidence. Other alternative explanations have been advanced (Vilter, 1938, 1939a,b; Umrath and Walcher, 1951). Gray (1956b) has suggested that "removal of central nervous control by nerve section might result in some inherent dispersing mechanism of the melanophore coming into play. Later the melanophores lose their refractoriness to diffusing neurohumours and may even become hypersensitive. This is a likely possibility since mammalian autonomic effectors become sensitized, not immediately but some time after their nerves have been sectioned."

Some other observations from Parker's laboratory give some support to the idea of double innervation. Thus Mills (1932a,b) produced caudal bands in *Fundulus*, allowed them to pale on a white background, transferred the fish to a black background and then, after some time, back to a white background. She observed that some of the melanophores on the edge of the band could disperse fully but not aggregate fully while others could aggregate fully but not disperse fully. This may indicate that in some melanophores only the aggregating fibers had been cut and in others only the dispersing fibers. Abramowitz (1935, 1936a) followed the growth of cut nerve fibers back to the melanophores and frequently tested the ability of the denervated melanophores to respond to black and white backgrounds. He found that melanophores may regain their power of rapid dispersion without being able to aggregate rapidly and vice versa. Parker and Rosenblueth (1941), working on *Ameiurus nebulosus*, claimed that electrical stimulation with higher frequencies and shorter pulses favored paling of the fish while lower frequencies and longer pulses favored its darkening (see Healey, 1940, 1951).

While there may be fibers causing active dispersion of chromatophores, their presence can not be regarded as proved. Young (1930) has shown that there is no real evidence for a sacral parasympathetic system in fishes and any observations in favor of double innervation of chromatophores obtained in the future would not demonstrate the existence of an autonomic system at all similar to that found in the mammals.

## VIII. THE FUNCTIONAL DEVELOPMENT OF THE NERVOUS SYSTEM

### A. Introduction

Many aspects of the functional development of the nervous system in fishes belong less to the present theme of fish physiology than to the field of more general nerve physiology and, as such, lie outside the scope of this chapter.

Most of the work which has been done has been concerned with neuromuscular mechanisms, since the movements of muscles are more easily observed and analyzed than the responses of other effectors. This topic has been reviewed by Barron (1941) and by Hooker (1952). Other references leading to the literature are quoted by Harris and Whiting (1954b) and Whiting (1955).

In addition there have been a few studies of the responses of chromatophores in early embryos. Reference to these may be found in Parker's monograph (1948).

The first movements of the somatic musculature shown by vertebrates have in many cases been considered to be myogenic, i.e. the result of some spontaneous activity of the muscles themselves. Some authors have disagreed with this. Thus Youngstrom (1938) considered that nerve fibers are present when the muscles show spontaneous activity. Hooker (1952) believed that a myogenic phase had been satisfactorily demonstrated in elasmobranchs but was not convincingly demonstrated for teleosts. According to him, the distinction made by earlier workers between myogenic, neurogenic, and reflexogenic activity was sometimes ambiguous. The movements have been thought later to become neurogenic, i.e. initiated by the nervous system, and, finally, reflexogenic, as the complete reflex arcs become functional.

### B. Cyclostomes

Leghissa (1943–1946) considered that the first movements made by *Lampetra* are reflexogenic. Harris and Whiting (cited by Whiting, 1955), however, concluded that the first movements, which are non-rhythmical, are not reflexogenic but they did not decide whether they are myogenic or neurogenic. The anatomical relations within the spinal cord of the young lamprey have recently been described by Whiting (1948).

### C. Elasmobranchs

Paton (1907, 1911) described rhythmical contractions of the trunk musculature of young elasmobranch embryos and considered them to be myogenic. Wintrebert (1920a,b) confirmed and extended Paton's observations. In early embryos of *Scyliorhinus canicula*, when only one branchial pouch had developed, he described side-to-side rhythmical movements of the trunk myotomes which were apparently spontaneous. These contractions took place independently (i.e. out of step) on the two sides of the embryo with a frequency and amplitude which remained practically constant for any given temperature and age of embryo. Wintrebert referred to these first contractions as "aneural", i.e. myogenic, and showed that after removal of the spinal cord the activity continued unaltered. In the developing embryo the first contractions are given by the more anterior myotomes, these being formed first. Later the movement is taken up by the more posterior myotomes. This development of function in an anterior-posterior direction is also seen as the later phases appear. As the embryo develops the individual rhythmical contractions of the two sides become coordinated and then reflexogenic.

Harris and Whiting (1954a,b) have confirmed Wintrebert's observations and have demonstrated the absence of nervous connections using histological methods. The right and left sides contract as two independent units, each with an almost constant frequency which is increased on both sides by acetylcholine. At the beginning of the next phase there are sudden changes in the frequency of the rhythmical contractions which are still independent of one another on the two sides of the embryo and accelerated by acetylcholine. Later these changes in frequency, which Harris and Whiting suggest are produced by participation of the nervous system, affect both sides simultaneously, although individual contractions on the right and left sides still remain uncoordinated. "These changes may be similar (both frequencies increase or decrease) or opposed (an increase in frequency on one side is accompanied by a decrease on the other); the phenomena clearly suggest the existence of central nervous excitation and inhibition" (Harris and Whiting, 1954a). Removal of the nervous system at this time is followed by a return to the regular slower myogenic rhythm with the two sides again fully independent of each other. This second phase thus appears to be neurogenic.

### D. Teleosts

There have been various accounts of the muscular behavior in teleost embryos. Tracy (1926), working on the toadfish (*Opsanus tau*), considered that the earliest movements were neurogenic but now believes them to be myogenic (Hooker, 1952). Tomita and Tuge (1938) studied *Macropodus*. Leghissa investigated *Salmo* (1941a,b; 1942). Coghill (1933), Sawyer (1944), and Hooker (1952) studied the killifish (*Fundulus heteroclitus*). Sawyer considered that early movements are myogenic because they begin when there is practically no cholinesterase present. Moreover, eserine does not affect the movements at this stage.

According to Whiting (1955) investigations on teleost embryos present certain special technical difficulties which help to account for the lack of available information. He described the early initiation and transmission of contractions as myogenic in the trout (*Salmo gairdnerii*), later stages being neurogenic and reflexogenic, respectively (see also Brinley, 1951).

## IX. VARIOUS TOPICS RELEVANT TO THE NERVOUS SYSTEM

There remain a few unrelated topics which concern the physiology of the nervous system but which cannot fit well into any ordered scheme.

### A. The Cerebrospinal Fluid

Van Rijssel (1946) investigated the circulation of cerebrospinal fluid in a species of carp (*Carassius gibelio*) by carrying out histological examinations after the animals had been allowed to swim for various times in solutions of trypan blue. The dye was absorbed through the gills, skin, and intestine and appeared in the blood but no trace was found in the central nervous system or its membranes. There thus appeared to be a blood-cerebrospinal fluid barrier for trypan blue. Injection of the dye into the pericerebral space and subsequent examination indicated that the blood vessels in the brain are accompanied by perivascular spaces communicating with the meningeal space and with the ventricular space and that all contain cerebrospinal fluid. This fluid is moved through the perivascular spaces by the pulse wave in the same direction as the blood stream and also through the thin roof of the third ventricle. Van Rijssel concluded that the relations in *C. gibelio* are in principle the same as those in higher vertebrates (see also Agersborg, 1948).

### B. Acetylcholine

Scattered references to the presence or action of acetylcholine in fishes occur in the literature. Reference may be made to Parker (1948), Jullien *et al.* (1950), Laurent (1952a,b), and the papers by Jullien and Ripplinger listed in the bibliography.

### C. "Moods," "Hypnosis," and General Inhibition

Conditions within the central nervous system which accompany certain "mental states" or "moods" in fish have been shown by von Holst and his co-workers to be reflected in certain aspects of visible behavior (von Holst, 1950; Schoen, 1949). Thus the angle at which certain fish orientate themselves with respect to gravity and horizontal illumination changes according to the state of rest or excitement of the animal, the latter being increased, for example, by the smell of food.

Ten Cate (1928b) confined *Raja clavata* to a restricted space and found that slight pressure with the hand on the head or back was generally followed by complete immobility of the animal from which it could only be aroused by relatively strong stimulation. He discussed these observations with reference to Pavlov's theory of sleep and cited earlier literature (ten Cate, 1928b, p. 190).

E. G. Gray (1956a) has described abnormal color responses shown by the minnow (*Phoxinus*) following inhibition of movement. If this fish is confined in a tube on an illuminated black or white background for some hours it becomes quiescent but can no longer become fully black or white adapted. Instead, it assumes an intermediate tint which it maintains indefinitely as long as it is undisturbed. An extraneous stimulus, such as movement in its field of vision, sudden change in the flow of water through the tube, or vibration, is followed by full darkening of the fish if it is on a black background. If it is on a white background the fish remains intermediate in tint. The abnormal tint associated with confinement appears to result from interference with the nervous chromatic mechanism and not the hormonal. Gray has suggested that confinement "abolishes continuous somatic motor activities, imposing a learnt inhibition on the central nervous system. It seems probable that this inhibition becomes generalized and affects the brain centers controlling color change. An extraneous stimulus appears to remove temporarily the central inhibition, permitting for a time a normal black background response."

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### CHAPTER II: PART 1

# THE SENSE ORGANS: THE EYE

J. R. BRETT

*Page*

I. Origin and Significance of the Eye . . . . . . . . . . . . . . . 121
II. Component Parts of the Visual System . . . . . . . . . . . . . 123
  A. Structure of the Eye . . . . . . . . . . . . . . . . . 123
  B. Structure of the Retina . . . . . . . . . . . . . . . . 126
  C. Optic Tract . . . . . . . . . . . . . . . . . . 128
III. Photoreceptive Functions . . . . . . . . . . . . . . . . 130
  A. Formation of the Image . . . . . . . . . . . . . . . . 130
  B. Photomechanical Responses . . . . . . . . . . . . . . . 134
  C. Process of Photoreception . . . . . . . . . . . . . . . 135
  D. Phototransmission . . . . . . . . . . . . . . . . . 141
IV. Functional Adaptations . . . . . . . . . . . . . . . . . 144
  A. Deep-Sea Adaptations . . . . . . . . . . . . . . . . 144
  B. Aerial Adaptations . . . . . . . . . . . . . . . . . 144
  C. Burrowing Adaptations . . . . . . . . . . . . . . . . 147
V. Synopsis . . . . . . . . . . . . . . . . . . . . . 147
  References . . . . . . . . . . . . . . . . . . . 150

## I. ORIGIN AND SIGNIFICANCE OF THE EYE

Light has been defined as radiant energy capable of stimulating the eye and causing the sensation of vision (Murray, 1952). In this physiological sense it cannot be measured by simple physical indices but must be indirectly determined for any particular animal by a measure of its "visible spectrum." The narrow limits of this spectral band of visual sensitivity in the total sweep of the radiant energy frequencies (Fig. 1) strongly reflects the point of origin of the vertebrate eye—in water. This fact defines many of the fundamental features of ocular anatomy and physiology, for the transmission spectrum of water is roughly the same as the visible spectrum, the point of greatest sensitivity in fishes corresponding to the wavelengths of greatest penetration (Clarke, 1936). The lack of sensitivity of the fish eye to infrared and ultraviolet radiations is proportional to the rapid extinction of these wavelengths in water.

Certain of the properties of light, that of traveling in straight lines, of varying in intensity and frequency, and of being partly reflected by most substances, make it ideally suited to fulfill the chief functions of any sense organ, to inform the animal of environmental changes and to provide a means of orientation (von Buddenbrock, 1952). In addition, through changes in intensity and day length, the visible spectrum gives rise to "light reflexes" which govern stages of internal development and excitation. Mediated by centers in the brain (hypothalamus and

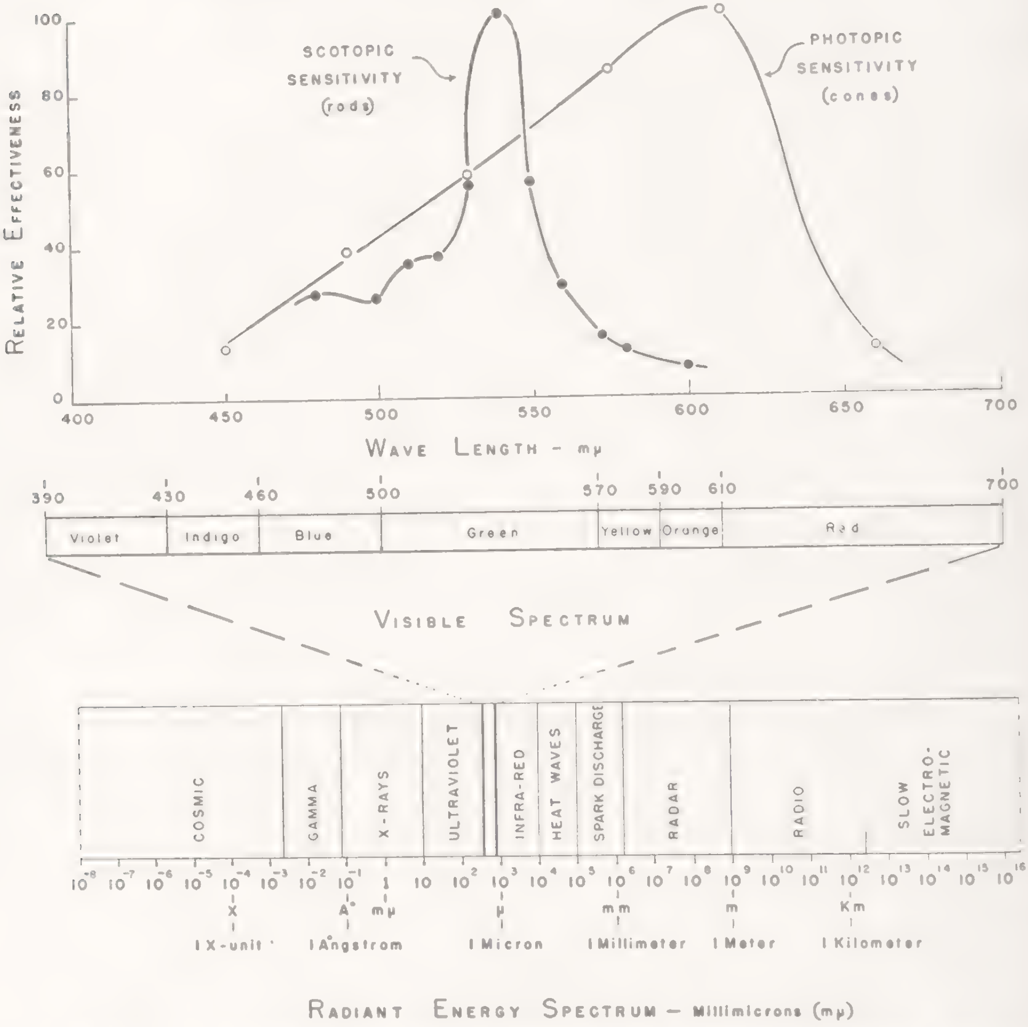

FIG. 1. The portion of the total radiant energy frequencies occupied by the "visible spectrum" of the sunfish, *Lepomis*. Scotopic or dim light sensitivity curve determined near the minimum threshold ($10^{-6}$ millilamberts) for 13 fish. Photopic or bright light sensitivity curve determined for 1 fish at 2 levels of brightness, several hundred times that of the scotopic level. Maximum sensitivity assigned a value of 100 in each relation. (Data for *Lepomis* from Grundfest, 1932a, b).

pituitary) light reflexes can influence the gonads (see Chaps. VI and VII, Vol I). Indeed, it seems likely that this "reflex" function is the more primitive from which vision has fortuitously arisen. Whatever the level of sight, the most ancient types of fossil fish, the Agnatha, such as *Cepha-*

*laspis* and *Pteraspis* (from freshwater Silurian and Devonian deposits) possessed distinct but not well-developed eyes, placed far apart on either side of the head.

Embryologically, the parts of the eye are derived from three sources, the ectoderm of the neural fold (retina, optic tract), the surface ectoderm (epithelial layer, lens) and the mesoderm (sclera, cornea, choroid, iris). From this it appears that no complex retina could have existed prior to the evolution of the internal tubular brain of the chordates. Walls (1942) draws attention to the presence of photosensory cells in the ventral "infundibular organ" of *Amphioxus*, a crude visual apparatus and possibly forming primitive homologues of the vertebrate rods and cones. The fossil record of the eye structure like that for most soft tissues is obscure. It can only be deduced that, through the thread of primitive Agnatha (cyclostomes) and less primitive selachians (elasmobranchs) and chondrosteans, the modern elasmobranch and teleost eyes have evolved.

The primary function of the vertebrate eye is to receive, resolve, and respond to light, transmitting the resulting stimulus to the brain. In some fishes like the plankton-feeding herrings (Clupeidae) sight is the dominant sense for seeking and selecting food (Vilter, 1950). This signifies the presence of an eye fully capable of detection of movement and appreciation of form, the necessary prerequisites for the effective discrimination of color.

## II. COMPONENT PARTS OF THE VISUAL SYSTEM [1]

The function of the eye is so intricately bound with its structure that it is impossible to consider the physiology without full regard to the anatomy. Although there are a great many variations within the major plan, the analogy between eye and camera has found increasing significance (Wald, 1950). It is the fact of having to meet common problems, those of focusing, of adequate but not excessive exposure, yet without distortion, that has resulted in convergence between the structural plan of the eye and the camera. Caution must be exercised, however, in extending this comparison since the eye has many diverse functions to perform and is adapted to meet a variety of changing circumstances (Smith, 1926).

### A. Structure of the Eye

Where fish can be shown to have at least moderately good vision, the eye is a comparatively large organ. Six oculomotor muscles, the standard number, provide synchronized rotation within the orbits. Eye movements are invariably coordinated, but some species like *Serranus* and *Blennius* show independent eye movements when converging on near objects. The typical eyeball (Fig. 2) is somewhat flattened on the anterior face.

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[1] Where greater detail is desired the reader is referred to the works of Wunder (1936), Walls (1942), Detwiler (1943), and von Buddenbrock (1952).

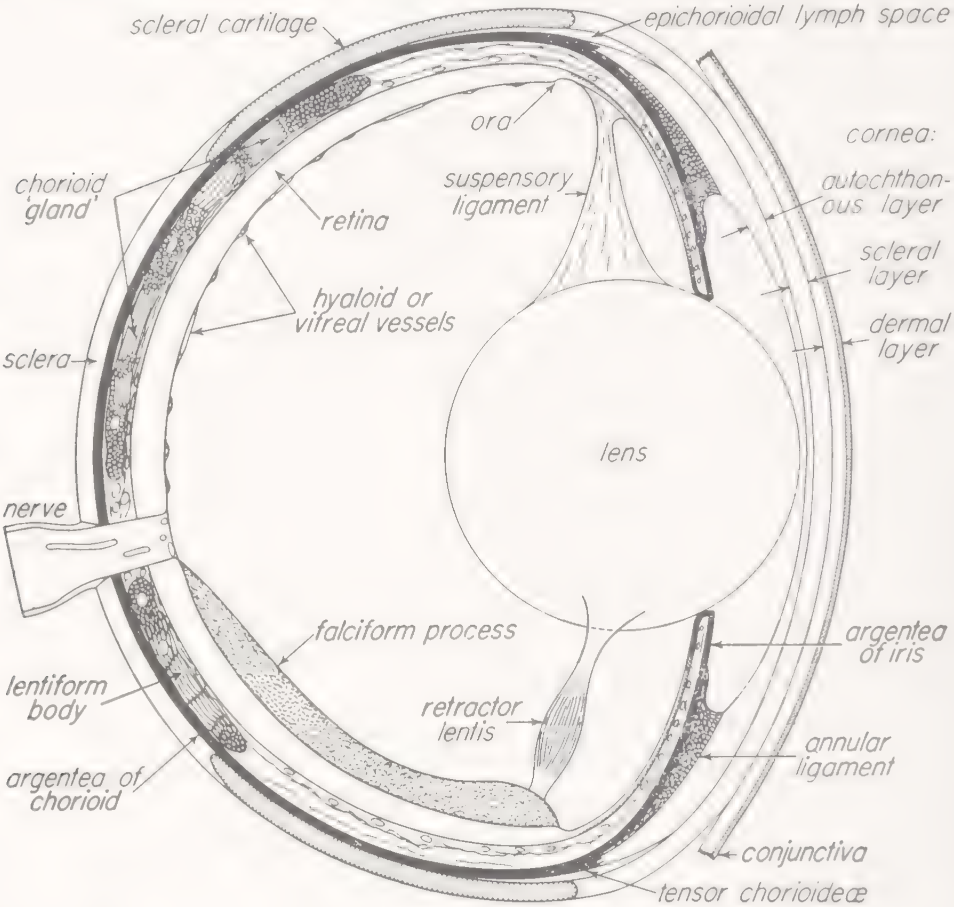

FIG. 2. Diagrammatic vertical section of a typical teleost eye. Not all structures shown are present in every teleost eye, e.g. hyaloid vessels are not present in conjunction with a falciform process (from Walls, 1942).

Its exposed surface, the cornea, is well streamlined in active fishes but may be irregular or concentrically ridged in others. There is no optical need for a smooth corneal surface, since its optical density is approximately that of water, and consequently of no refractive importance. The cornea is an avascular tissue obtaining its nourishment from the intraocular fluid (Krause, 1934). A portion of its oxygen supply must come from the surrounding water. Among aquatic and hibernating mammals (Duane, 1949, Langham, 1952) the cornea is resistant to low oxygen and the cornea of a fish is probably similarly endowed.

The transparent cornea is continuous with a tough, flexible, opaque envelope, the sclera, which resists changes in shape as a result of distention from an internal ocular pressure and the presence of an encircling band of cartilage. Between the cornea and the iris, and enveloping the forward third of the lens, is the anterior chamber. It is filled with a somewhat saline solution, the aqueous humor. Behind the lens is the posterior chamber which in turn is filled with the vitreous humor, an almost gelatinous matrix owing to the presence of proteins secreted by the retina during development. Blindness has been produced experimentally in some fishes by injecting this chamber with phemerol, causing degeneration of the eye (Walker and Hasler, 1949). This two-fluid system, although persisting throughout higher vertebrates, is of greatest importance in the fish eye where focusing is achieved by advancing or retracting the spherical lens. Free movement is permitted within the anterior chamber, whereas the viscous vitreous humor helps to prevent the lens from being displaced into the larger, posterior chamber.

Fringing the edge of the lens is the iris, a pigmented, richly vascularized disc. Its central opening, the pupil, is immobile in some fish: those in which the iris is devoid of muscles (e.g. *Lepisosteus*) and others with a noncontractile sphincter. A few teleosts such as eels, flatfishes, and stargazers, show considerable pupil movement (Young, 1931, 1933b). Most elasmobranchs exhibit extensive but slow contraction of the iris, requiring two to three minutes to close in bright light and at least an hour to reopen in the dark (Young, 1933a). In nocturnal elasmobranchs the contracted pupil may take the form of a horizontal or vertical slit. Much of the iris is covered externally by a very ductile annular ring (annular ligament) linking the iris to the cornea. There is some doubt as to the function of this tissue but its secretory appearance has led Walls (1942) to suggest that it is possibly the source of the aqueous humor.

The lens, made up of concentrically formed layers, is an isolated transparent colony of cells without a nerve or blood supply. In teleosts, it is attached dorsally by the suspensory ligament. Ventrally a retractor muscle (retractor lentis) pulls directly on the lens drawing it backward and temporad. This focusing muscle is attached to the distal end of the falciform process, a sickle-shaped prominence characteristic of most teleosts (not found in elasmobranchs) and extending as a ridge from near the iris backward into the fundus or pit of the eye. Where it is absent (e.g., eels, puffers, anglers) there is a system of hyaloid blood vessels distributed along the inner surface of the retina, clearly suggesting a nutritional function for the alternative falciform process.

Distributed between the sensitive retina and the protective sclera, and continuous with the iris, is the choroid. Two attributes of the choroid (vascularization and pigmentation) define its major functions. The oxygen demand of the highly metabolic retina is serviced by a multitude of blood vessels, the choriocapillaris, as well as a mass of capillaries known as the choroid gland forming an arc around the optic nerve. The gland probably serves to smooth out blood pressure pulses from the heart, reducing mechanical disturbance of the retina (Walls, 1942). The particularly well-developed choroid capillary system in the eye of the lingcod (*Ophiodon elongatus*), commonly found to range from shallow to deep water, is considered by Allen (1949) to be an adaptation to pressure change. This contention is supported by the presence of an alternative route in the venous return flow, either "by way of myriads of very fine rete mirabile under possibly increased pressure, or by much coarser dorsal and ventral choroid veins under a low pressure." Duke-Elder and Davson (1948) in dealing with the general problem of intra-ocular pressure point out that the driving force of fluid into the eye comes from capillary pressure plus an excess of osmotic pressure. The intra-ocular pressure must be less than the capillary pressure in order to allow free capillary flow. Drainage from the eye chambers accounts for the pressure differential. Aside from the melanin pigmentation of the choroid which serves to prevent internal reflection, there is usually a guanine layer, the argentea, next to the sclera. In the early stages of development, guanine coats the choroid concealing the melanin already present and prominent in the transparent body of the larva. It loses most of its protective significance in later stages and is seldom found in deep-sea fishes. Guanine deposits may also act as a mirror serving to increase the stimulus resulting from dim light by reflecting the transmitted light back through the retina. Such a deposit of guanine may be located in the choroid, where it is called the tapetum lucidum (elasmobranchs and some marine teleosts), or within the pigment epithelial cells of the retina (a few cyprinids and percids, and many bathypelagic species).

### B. Structure of the Retina

The retina is the seat of photoreception. It is made up of a complex series of cells distributed in four interlocking layers (Fig. 3A, B). The cells are all derived from or are characteristically nerve cells, reflecting the embryonic origin of the retina as an outgrowth of the developing central nervous system. Like all nerve tissue it is highly sensitive to oxygen lack. Using a Warburg respirometer Lindeman (1943) has studied the oxygen consumption of retinal cell suspensions in three species of teleosts. The rate of uptake for the chub (*Leucosomus*) and shiner (*Notemigonus*) was approximately 0.76 mm.$^3$ mg. hour, while that of the sucker (*Catostomus*) was only 0.59 mm.$^3$mg. hour.

Covering the outer circumference of the cup-shaped light receptor and immediately adjacent to the choroid is a pigmented epithelial layer which enhances the light-absorbing qualities of the choroid. These cells, containing fuscin and sometimes guanine, usually have long thick

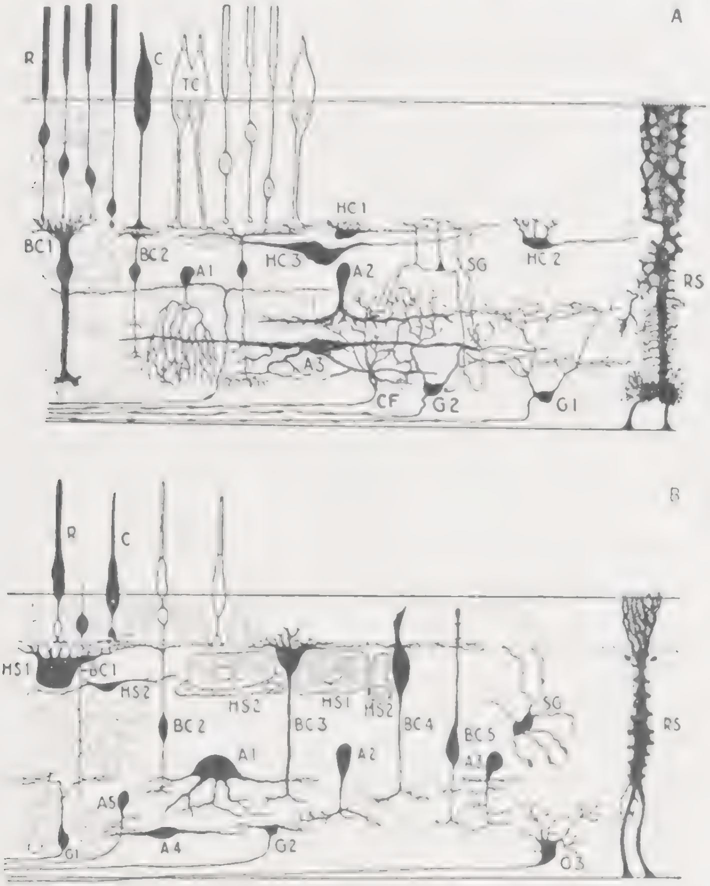

FIG. 3. Diagrammatic vertical section of (A) teleost retina and (B) elasmobranch retina. From Detwiler (1943) after Franz, 1913. A 1–5, amacrine cells; BC, 1–5, bipolar cells; C, cone; CF, centrifugal nerve fiber; G 1–3, ganglion cells; HC 1–3, horizontal cells; HS 1–2, horizontal supporting cells. R, rod, RS, radial supporting cell; SG, stellar ganglion cell; TC, twin cone.

processes enveloping portions of the photosensitive rods and cones. Needle-shaped granules of the pigment are freely mobile and serve on occasion to mask the photosensitive rods from excessive light.

The rods and cones containing the photosensitive pigments in their outer segments, together with their nuclei and fibers, form the next layer—the receptor layer. Three types of visual cells occur in the teleost retina: rods, single cones, and twin cones. All three types are not present throughout the retina, nor in all species. Pure rod retinas occur in deep-sea teleosts and in most elasmobranchs except the smooth dogfish, *Mustelus*, and the eagle ray, *Myliobatis*. These elongated cells, arranged in neat mosaics, actually point away from the light. This anatomical relation is unlike that of most invertebrates and necessitates high transparency of the remainder of the retina through which the light must pass to reach the visual elements. Such transparency is reminiscent of the larval stage in which most tissues are transparent.

The second link in the chain of visual neurons is formed by the bipolar cells (third layer histologically). They constitute an intermediate connection between the visual cells proper and the ganglion cells (fourth layer). Dendrites from the bipolar cells connect with the cones singly, or with groups of rods collectively, and are in turn interlocked with dendrites from the ganglion cells. These multiple-synaptic junctions serve to transmit the visual impression along the grouped axons of the ganglion cells, radiating towards and making up the fibers of the optic tract.

If the visual-bipolar-ganglion cell arrangement represents transmission through the retina vertically, then lateral communication is performed by the horizontal and amacrine cells. These latter cells have a similar role to the bipolars and may well be special modifications of them. It is the presence of this intricate network, linking visual cells and inter-relating ganglia that makes the visual process a very complicated phenomenon. Increasing acuity can be associated with increasing complexity of the retina as much as with development within the optical centers of the midbrain itself.

## C. Optic Tract

In the past the optic tract has usually been called the optic "nerve" with the term "tract" reserved for that portion of the tract within the cranial chamber where the two cross over (optic chiasma) and continue into the brain. Young (1950) stresses the fallacy of this nomenclature, which, if not appreciated, could lead to functional misconceptions. The retina, as described, is an outcropping of the brain and connected with it by bundles of axons linking neurons of the central nervous system. A number of true nerves service the eye and its musculature, namely the oculomotor, trochlear, abducens, and branches of the trigeminal (Fig. 4). Constriction of the iris of elasmobranchs results from activation of the sphincter iridis muscle by direct illumination; its dilatation, however, is motivated by motor fibers from the oculomotor nerve (Young, 1933a, c). In a few teleosts, e.g. *Lophius* and *Uranoscopus*, the iris is contractile and constriction is mediated by sympathetic nerve fibers (Young, 1933b). The relative development of the optic tracts and lobes in a variety of teleosts was shown to be greatest in surface-feeding fish which are predominantly dependent on visual discrimination (Mookerjee, Ganguly, and Mookerji, 1950).

Sectioning of the optic tract for blinding does not always result in degeneration (Sperry, 1949). Rasquin (1949) has shown that if the cut ends approximate one another in the wound, and vascularization is established, regeneration may occur. An interesting method of monoc-

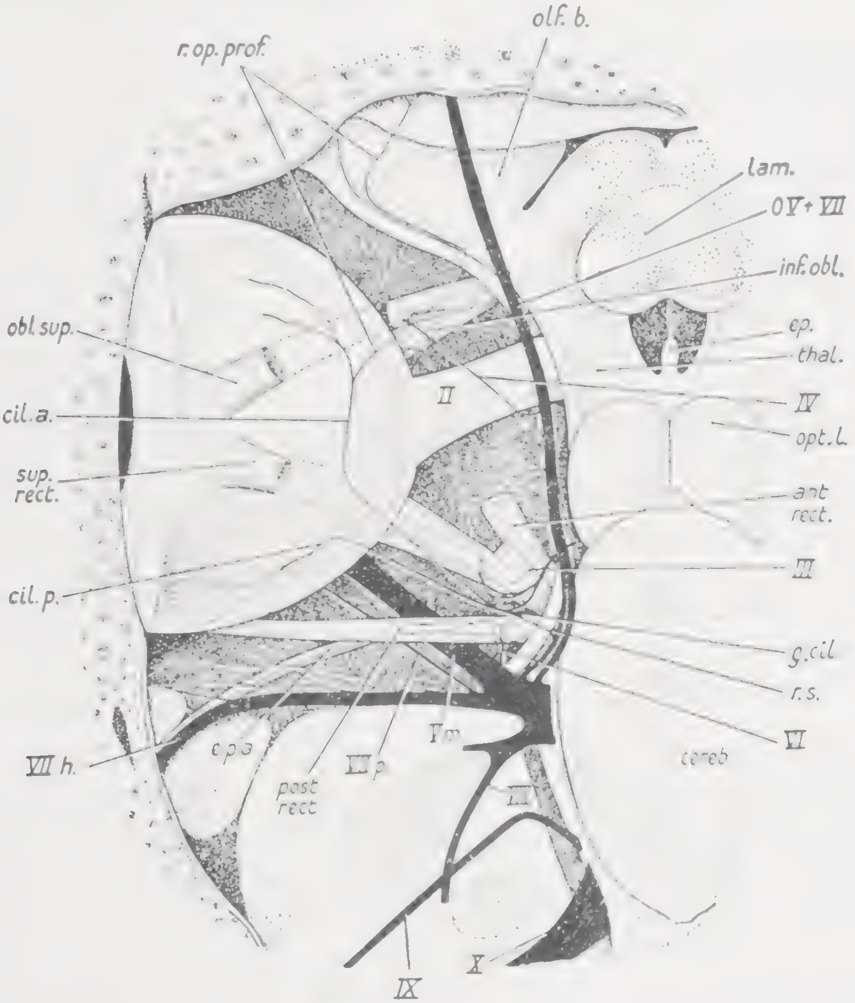

FIG. 4. Orbit of the dogfish showing innervation of the eye and its relation to the brain (from Young, 1950), ant. rect., anterior rectus; cereb., cerebellum; cil.a., cil.p., anterior and posterior ciliary nerves; epa. efferent pseudobranchial artery; ep., epiphysis; g.cil., ciliary ganglion; inf. obl., inferior oblique; lam., lamina terminalis of cerebrum; O.V + VII, superficial opthalmic branch of trigeminal and facial; obl. sup., superior oblique; olf. b., olfactory bulb; opt. l., optic lobe; post. rect., posterior rectus; r. op. prof., ramus ophthalmicus profundus of trigeminal; rs., sensory root of ciliary ganglion; sup. rect., superior rectus; thal. thalamus; II to X, cranial nerves.

ular blinding is described for the goby (*Bathygobius soporator*) by Sperry and Clark (1949). An opaque cap of tantalum foil was inserted through an incision in the sclera just dorsal to the cornea and maneuvered to cover the pupil. After shifting the blinder, retention tests of habits induced by training and dependent upon vision revealed good interocular transfer in about one-third of experimental cases (and see Chap. I).

## III. PHOTORECEPTIVE FUNCTIONS

Light, as it passes from a less dense to a more dense medium, is refracted, i.e., the "rays" are bent toward a perpendicular to the interface. The greater the "optical density" the greater the refraction, which, when expressed as the ratio of the speed of light in air to that in the new medium, is called the "index of refraction." As a result of a combination of refraction, reflection, and absorption of light in water, the amount of light available to a fish's eye is greatly reduced from that in air, particularly so with increasing depth or turbidity. In water, particularly salt water, the multitude of small suspended particles and free-living organisms reflects light from all angles with the result that shadows are diffuse and objects at a distance are "diluted" (Reighard, 1917). Reflected light from below, which reaches the water-air interface at an angle of 48.8 degrees or less, is internally refracted, creating a mirror-like surface screen. Vision of objects in air is greatly restricted below a depth of a few meters even when the surface is relatively undisturbed by wave action. The task of the fish eye is to collect sufficient light from as many directions as possible and to resolve this into distinguishable shapes, sizes, and patterns.

### A. Formation of the Image

The optical density of the cornea, aqueous humor, and vitreous humor, all approximate to that of water (refractive index, 1.33). Consequently the role of image formation falls entirely to the lens in which a combination of high optical density and spherical shape leads to great resolving powers and a very short focal distance. This has permitted the optical axis of the eyeball to be its shortest diameter and, in teleosts, the unaccommodated point of distant focus converges in front of the retina (myopia). Since near vision must be the more frequent demand on the eye, the tendency toward myopia seems quite in keeping with its use.

The firmness of the lens of a fish does not permit flexing the sphere to alter the surface relations for focusing, as in the case of higher vertebrates. Instead the retina–lens distance is altered. This is achieved in teleosts by movement of the lens through contraction of the retractor lentis, the fluid nature of the aqueous humor permitting backward displacement within the anterior chamber. The lens when relaxed is advanced almost in contact with the cornea which itself is somewhat bulged outwards. It is quite possible to observe a portion of both lenses in such pelagic fish as the herrings (*Clupeidae*) from a point immediately above the head, indicative of the characteristically wide field for periscopic vision.

Accommodation by shifting the lens is also characteristic of the elasmobranchs, but the eye is basically hypermetropic (far-sighted) and the accommodating muscle, the protractor lentis, has a different origin from that in teleosts. In bright light the elasmobranch has the advantage of increased acuity through full contraction of the iris, producing a fairly sharp, pin-point image regardless of distance.

It is interesting to note that lampreys (e.g. *Lampetra*) employ an entirely different method of accommodation. A transparent area of the skin, known as a "spectacle," covers the cornea. It is attached at its posterior edge by the cornealis muscle which, when contracted, causes the spectacle to flatten thereby exerting an inward pressure on the lens, displacing it towards the retina and bringing more distant objects into focus. Walls (1942) records that the near-point of focus for the resting eye is at about 5 inches.

Following the investigation of some 68 species of teleost, Beer (1894) recorded movement of the lens by electrically stimulating the retractor. Despite the current acceptance of this functional relation (Meader, 1936; Walls, 1942; Young, 1950; von Buddenbrock, 1952), Verrier (1948) has disputed the mechanism of accommodation. Verrier has denied the presence of muscle fibers in the retractor lentis (or campanula Halleri) and states that the teleost eye (carp, tench, and roach) is markedly hypermetropic, and that accommodation results from the contraction of fibers in the ciliary body, as in most vertebrates. This contraction does not, however, affect the inflexible lens but rather it increases the anterior-posterior diameter of the eye by deforming the ocular globe. The presence of serous pockets in the choroid makes this unique method possible. Further work by Baron and Verrier (1951) on 25 species of teleosts and selachians supports their contention that hypermetropia characterizes both groups. In addition, measurements of irregularities in the curvature of the lens demonstrated the presence of a considerable degree of astigmatism. The combination of moderate hypermetropia, even when accommodated, and astigmatism, coupled with a large number of visual cells in relation to ganglia, has led Verrier (1948) to conclude that the teleost eye is basically suited for detection of movement and is defective in the perception of form.

Many authors (e.g. Wunder, 1936; Meader, 1936) have warned against generalization about the eyes of fishes since they show so many diverse and multiple modifications. Obviously all fishes will not have or need equally good vision. That exceptionally acute vision has been developed in some species is shown by the habits of the East Indian archer fish (*Toxotes jaculator*) which accurately strikes down insect prey in the air by ejecting a fluid projectile from a distance of 3 to 5 feet (Schultz and Stern, 1948).

Precise focusing of an image on the retina is a prerequisite to clear vision. It might be noted, however, that since the photosensitive portion of the visual cells has *length*, the point of focus can fall anywhere within this range and still be precise. The length of the outer segment of the rods and cones in fish ranges from 3 to 40 $\mu$ (Osborn, 1935; Detwiler, 1943; Moore and McDougal, 1949).

A further factor which governs acuity is the concentration of visual cells per unit area of retina and the number of ganglia supporting these. The finer the "grain" of the retina the greater its ability to distinguish the details of visual objects. Allen (1951) illustrates this point well in man by a comparison of the distance between sensory endings in the skin and retina. The minimum distance apart that two points can still be detected on the retina is $^{1}/_{3000}$ that of the skin. Wunder (1925, 1926, 1936) determined the number of rods and cones in the eyes of various fish. The greatest concentration was found in the crepuscular eel-pout, *Lota lota*, in which numerous extremely small rods (810,000 sq. mm.) outnumbered the cones (3400 sq. mm.). Such a concentration, however, does not signify acuity since Wunder (1936) further reports that on the average 263 visual elements interlock with 7 bipolars which connect with but 1 ganglion cell. The crepuscular eel-pout is obviously adapted for vision under conditions of low illumination. *Ameiurus nebulosus* which hunts in good light had the largest concentration of cones (9000 sq. mm.) in contrast with the almost complete lack of cones in deep-sea fishes.[2] The stout rods of the rather inferior eye of the catfish, *Ameiurus nebulosus*, (Welsh and Osborn, 1937) were numerically among the least concentrated (18,400 sq. mm.). Cone high retinas characterize fish that feed in bright light and have good vision, whereas large and numerous rods are associated with life in low illuminations. This differentiation of function between rods and cones has been found throughout the vertebrates. High sensitivity to light is the property of the rods (Hecht and Mintz, 1939; Detwiler, 1943), groups of which are

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[2] Not supported by Detwiler, 1943, p. 94. "In deep-sea fishes where one would expect rods to prevail, they apparently are fewer (Karsten, 1923) or are lacking altogether (Brauer, 1908)."

linked with single ganglion cells, thus increasing sensitivity at the cost of acuity. In bright light the stimulus is greater and the sensitivity of the retina can be sacrificed for increased acuity. A predominance of cones in particular areas of the retina has been shown to be correlated with feeding habits requiring acute vision. From sections of the sardine eye, *Sardina pilchardus*, Vilter (1950) has related the high cone-rod ratio (8 to 1) of a segment in the ventral portion of the retina with the ability to feed selectively on small crustaceans from among a mass of plankton. The poorly swimming, bottom-feeding fish, *Callionymus lyra*, shows differentiation in the appropriate *dorsal* half of its retina. The guppy, *Lebistes reticulatus*, has a double area in its retina (Vilter, 1948) associated with an increased number of bipolar cells which exceed the photoreceptors in number. The numerical predominance of the bipolar cells is greatest in the ventral area, on which the image of surface food particles would be cast. The function of the bipolars probably includes elaboration as well as association of visual messages.

In a few fish a further modification is found at the retinal point of focus of objects in the forward field of vision. These have a pit-like depression or fovea, with a locally increased concentration of cones. The shape of the depression, with its walls arched inwards, and the slight refraction of light rays passing through the optically more dense retinal layers, would magnify the image. The foveated fishes are all marine species inhabiting the littoral zone and mainly of the genera *Serranus*, *Syngnathus*, and *Blennius* (Kahmann, 1936, Baron and Verrier, 1951). Another unique attribute of these foveated fishes is the ability to converge both eyes independently in temporary coordination on an object in the narrow anterior field for binocular vision. Normally the eye movements of fish are controlled by reflexes stemming from the inner ear which tend to maintain the eye in a level position (Lowenstein, 1937; and Chap. II, Part 2).

Senders (1948) defines visual acuity as the "reciprocal of the minimum visible angle measured in minutes of arc," pointing out that the definition is in terms of angles based on the implicit assumption that visual acuity is independent of distance (other things being equal). It is impossible to determine the anatomical or physiological factors which *limit* acuity until maximum acuity has been measured. Senders further notes that at one time the size of the foveal cones was thought to be the limiting factor but measures of much finer acuity have since been obtained. Limits of discrimination in fish have been studied by Rowley (1934). Cyprinids were able to distinguish between circles, squares, and triangles. Goldfish differentiated between stripes 10 mm. and 2 mm. wide but not between 10-mm. and 8-mm. stripes. Their limit of discrimination between circles under optimal illumination was found to be a 0.3 cm. diameter difference from a standard circle 3.0 cm. in diameter, viewed from an average distance of about 8 cm. Mast (1915) reports that flounders (*Paralichthys* and *Ancylopsetta*) showed a differential response between dots 2 mm. and 1 mm. in diameter.

### B. Photomechanical Responses

The almost mutual exclusion of acuity and sensitivity has been one of the major problems in the development of a versatile eye. Various adaptations within the retina help to meet the dilemma. In particular the migration of pigments which mask the rods in bright light, with accompanying movement of the visual cells, is well developed in many fish such as *Ameiurus*, *Abramis*, and *Fundulus* (Arey, 1916a, b) but not in the eel (see Detwiler, 1943). In the absence of a contractile iris the mobility of the visual elements is all the more important to teleosts.

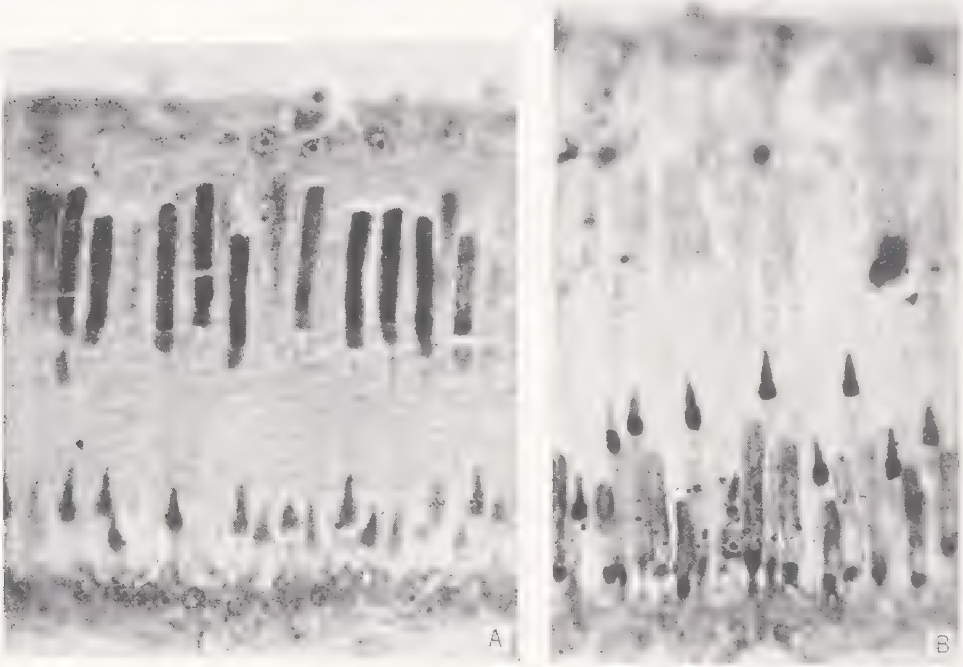
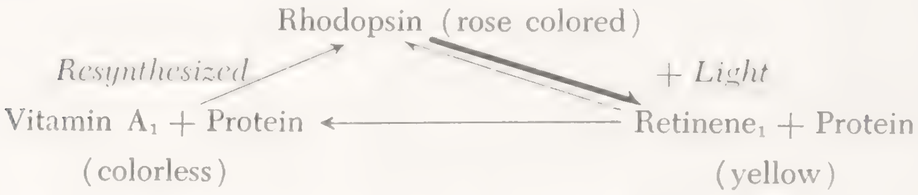


Fig. 5. Photomicrograph of a depigmented section of the retina of a catfish (*Ameiurus*) showing visual cell migration. A: *Light adapted*, cones advanced, rods retarded. Kull's stain × 500. B: *Dark adapted*, rods advanced, cones retarded. Iron hematoxylin × 500. (From Welsh and Osborn, 1937.) It is conventional to show sections in this "inverted" manner, with the line of normal light penetration proceeding from below through the transparent (here stained) ganglion and bipolar layers.

Pigment migration occurs along the length of the retinal epithelial cells, which extend almost to the external tips of the photosensitive portions of the rods and cones (receptor layer, terminated by the external limiting membrane). When exposed to light, a large portion of the retinal pigment moves into the pigment cell processes, surrounding and shielding the rods (e.g. *Phoxinus* in Wunder, 1936). There is a simultaneous movement of both rods and cones as a contractile portion of the cell body, the myoid, alters the length of the visual cells (Fig. 5A, B). The cones contract forward, while the pigment envelops the retreating rods. Arey (1928) records that the myoid may lengthen as much as ten times its contracted dimension. In dim light or darkness the reverse process occurs: the pigment granules become concentrated in the cell bodies of the retinal epithelium, and the rod myoids contract bringing their photosensitive endings forward beyond the elongating cone myoids. The pigment movements are not rapid, requiring up to an hour for migration whereas considerably less time is necessary for complete visual cell movement. According to Walls (1942) the single and twin cones of fishes migrate at different rates and to different extents. Vilter and Thibault (1948) have illuminated a segment of the dark-adapted carp eye and found that pigment migration was more pronounced in the lighted area. Some response also occurred in the unilluminated eye, supporting the earlier conclusions of Arey (1916a) that retinal pigment and visual cell migration are at least partially under the control of the central nervous system. A persistent diurnal rhythm in the movement of rods and cones in complete absence of light has been observed in *Ameiurus* by Welsh and Osborn (1937) and Arey and Mundt (1941). This has lasted up to four days, diminishing greatly in magnitude with time.

## C. Process of Photoreception

The essential factor in sensitivity to light is a chemical change. Stimulation of the sensory part of the retina by the light of the focused image causes a chemical decomposition of photolabile substances within or near the tips of the rods and cones (Murray, 1952). The products of decomposition stimulate the receptors to initiate a series of nerve impulses culminating in sight. In spite of the wide anatomical differences in image-forming eyes, not only among vertebrates but also arthropods and molluscs, the photochemical processes operate within a very limited group of chemical substances and a restricted pattern of reactions. In the rods of almost all vertebrates the specific photosensitive substance, rhodopsin (visual purple), has been known since the time of Boll (1876) and its chemistry was extraordinarily well worked out by Kuhne in 1879 (Wald, 1945). It has never been found in other tissues than the retina. Rhodopsin, which may be extracted into aqueous solution by surface-active detergents such as digitonin and saponin, is a rose-colored (spectral absorption peak at 500 $m\mu$) conjugated protein in which the prosthetic group is a carotenoid closely related to vitamin A. On exposing the retina to light the carotenoid is split off from the protein and goes through a succession of changes to form a more stable yellow pigment, retinene$_1$ (387 $m\mu$). Further exposure results in bleaching to the colorless vitamin A$_1$ (328 $m\mu$). In an intact animal the retinal rhodopsin is resynthesized from protein and vitamin A$_1$ in the dark (Florkin, 1949). The result is a closed cycle of the form: [3]



This is readily demonstrated among marine fishes (Wald, 1945).

Not all wave-lengths in the visible spectrum are equally effective. This simply means that the photosensitive pigments of the rods and cones have absorption spectra which are not uniform. The more efficiently light of a given wavelength is absorbed, the greater the stimulus for equal energy emissions. The fact that different wavelengths can create differing degrees of brightness sensation, aside from any color sense, and the differential absorption of light as a result of either different pigments or diverse filters in the eyes of vertebrates, constitutes the physical basis for the difficulty in determining color sense in animals.

The rhodopsin retina is characteristic of most marine fishes and almost all terrestrial vertebrates. Freshwater fish form a marked exception to this rule. They possess a purple rather than rose-colored retina in which rhodopsin is replaced by porphyropsin (Wald, 1937). The porphyropsin or visual violet system duplicates the rhodopsin cycle in every respect except that the constituents have absorption spectra consistently displaced about 20–30 $m\mu$ towards the red, or longer wavelengths (porphyropsin, 522 $m\mu$; retinene$_2$, 405 $m\mu$; and vitamin A$_2$, 355 $m\mu$). Bayliss, Lithgoe, and Tansley (1936) and Wald (1939a, b, 1941, 1945) have studied the distribution of the two pigments in a wide variety of bony and cartilaginous fishes. Among the marine fish studied by Wald (1945) the only exception to the presence of rhodopsin occurred in the family of wrasses (Labridae, e.g., *Tautoga onitis*) which contained a prevalence of porphyropsin in their retinas. The anadromous and catadromous species possessed predominantly one or other of the two systems, the predominating pigment being that normally associated with the salinity of their spawning environment -rhodopsin, saltwater; porphyropsin, freshwater (Fig. 6). This relation is also shown by the sea lamprey, *Petromyzon marinus*, which spawns in fresh water and has a predominance of vitamin $A_2$. Florkin (1949) comments that "it would be of great biological importance to discover if the porphyropsin retinal system had any survival value in the freshwater environment, or was a purely fortuitous innovation in the adventures of the evolutionary process." Examination of pigment extracts from dark-adapted fish retinas has led Dartnall (1952a, b) and Crescitelli and Dartnall (1954) to conclude that the main visual pigment in some freshwater fish occurs in at least two forms: one, with a maximum absorption peak at 533 mμ, found in tench (*Tinca*), pike (*Esox*), and bleak (*Alburnus*), and a second, in the carp (*Cyprinus*), with a maximum at 523 mμ. In the tench and bleak a small amount of other pigments was also present. The authors do not suggest that their results conflict with Wald's concept of two distinct systems predominating in fresh and saltwater fish, although vitamin $A_1$ appears to occur more frequently in marine species (Collins, 1954) than Wald had earlier suspected.

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[3] For a thorough consideration of the chemical aspects of the visual cycle see Collins (1954).

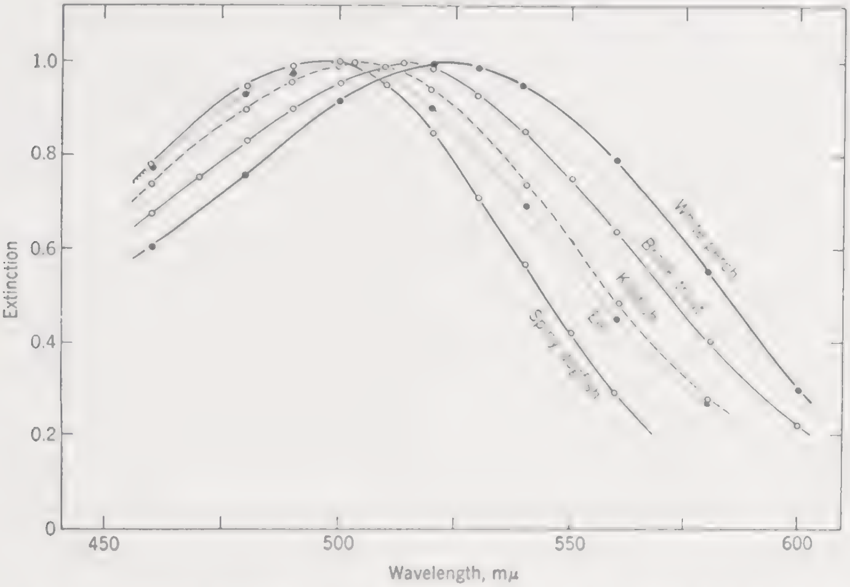

FIG. 6. Absorption spectra of photopigments from various fish illustrating the transition from a typically rhodopsin system (dogfish) to an exclusively porphyropsin system (white perch). Intermediate stages possess both pigments in varying degrees of predominance (from Wald, 1941).

Our knowledge of the chemistry of cone vision is singularly lacking.

Only recently has direct evidence for low concentrations of analogous pigments in the cones been reported (Wald, 1945; Bliss, 1946), though not in fish. A violet pigment called iodopsin has been extracted from chicken retinas. Since cone vision is functional under conditions of bright light the photo-labile system must be relatively stable yet sensitive to different qualities of light. Where the ability to distinguish different wavelengths of light of equal brightness (color sense) has been demonstrated it has invariably been associated with the presence of cones.

The measurement of visual perception depends on responses given by an animal following movement or intensity change in its visual surroundings. Hecht and Wald (1934) outlined clearly the basis for any experimental approach. They state that, "Presented with a visual field composed of a definite pattern, an animal can obviously respond to a movement of this pattern only when it is able to resolve the essential elements of the pattern. The composition of the pattern may then be varied to obtain a measurement of either visual acuity or of intensity discrimination. The simplest pattern is a series of stripes. For visual acuity the stripes may be varied in width and the intensity determined at which they are just resolved. For intensity discrimination the relative intensities of the alternating stripes may be varied and the minimum difference in intensity determined, which, for a given stripe width, will just elicit a response at a series of selected intensities." By the use either

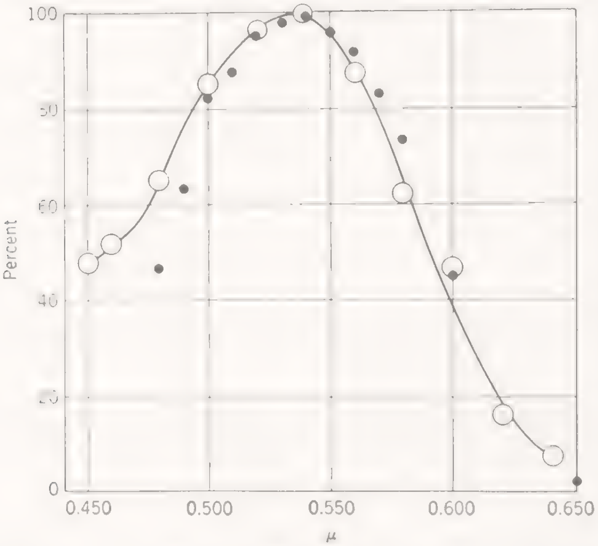

FIG. 7. **Comparison of scotopic sensitivity curve of the tench determined by** microelectrode technique (open circles) and absorption spectrum of retinal photopigment, porphyropsin (solid circles). Spectral absorption points from Bayliss, Lythgoe, and Tansley (1936). From Granit (1941, 1947).

of color filters, a spectrometer, or a monochromator, spectral lights of known wavelength can be obtained and the range of sensitivity throughout the visible spectrum defined. The difference in response results solely from brightness discrimination, a subjective experience in the central nervous system of an intact animal; it is not dependent on any color sense.

Sensitivity of the retina *per se* has been explored by microelectrode techniques picking up impulses in the optic fibers when the retina is illuminated. Granit (1941, 1943, 1947) has used this method to determine the dark-adapted sensitivity curve of the tench, *Tinca* (Fig. 7), and has compared this with the spectral absorption curve of extracted visual purple determined for this species by Bayliss, Lythgoe, and Tansley (1936). There is a slight difference between the two curves toward the violet end of the spectrum (between 450 and 500 m$\mu$) and Granit attributes this to possible fluorescence produced within the eye or to the presence of another photochemically active substance.

Physiological evidence for the existence of a duplex visual system (Duplicity Theory) in fish has been demonstrated by Grundfest (1931, 1932a, b) and Crozier and Wolf (1940). Grundfest examined the spectral sensitivity of the sunfish, *Lepomis*, under dim and bright light. The experimental procedure involved determining the minimum intensity of a given spectral band which just elicited the pseudo-rheotropic response to a slowly rotating screen. At low illumination of the order of $10^{-4}$ millilamberts, and using bars 2 mm. wide, the wavelength of maximum efficiency lay between 535 and 545 m$\mu$ (Fig. 1), approaching the position of maximum absorption for porphyropsin. The discrepancy in this case was explained by assuming that other light-absorbing pigments were present and acted as filters. Several such eye pigments are common, e.g. carotene, guanine, melanin. Grundfest (1932a) also noted that the cornea of the sunfish was of a grayish green color. A few fish, e.g. *Amia*, *Esox*, and *Cyprinus*, have yellow corneas (Walls, 1942). Visibility curves similar in form to those obtained by Grundfest have recently been described by Kawamoto and Konishi (1952) for two freshwater species, *Aplocheilus (Oryzias) latipes* and *Sphaeroides rubripes*, with maxima at about 510 and 500 m$\mu$.

After raising the intensity by 100 to 200 times that of the dim light or scotopic experiments, Grundfest (1932b) demonstrated that maximum sensitivity occurred at about 600 m$\mu$ in *Lepomis* showing a shift toward the red end of the spectrum. Granit (1941) has found similar peaks of photopic sensitivity in *Cyprinus* (600–610 m$\mu$) and *Tinca* (600 m$\mu$). The change in spectral sensitivity which accompanies increasing incident light on the retina is known as the Purkinje phenomenon and may be used to determine the light intensity which marks a shift in predominance from rod to cone vision (Hecht and Mintz, 1939). This occurs at an incident intensity of between 1.0 and 0.1 millilamberts for *Xiphophorus* (Crozier and Wolf, 1939).

The simple division of retinal functions into two distinct systems implied by the Duplicity Theory is not considered tenable by Escher-Desrivières and Verrier (1951). From their own investigations on over 200 vertebrates, including a comparative study of the rather rare foveated fishes (e.g. *Julis giofredi*), and finding support from the work of Granit (1947), they postulate a continuous series of cell types and consider that daylight and color sensitivity are independent of the visual cell type. Instead, photopic or bright light vision is a product of the modal character of activity of the photosensitive elements in which rod-like cells play a functional role. Granit's electro-physiological division of the elements into three groups responding to *on*, *off*, and *on/off* illumination could be a product of the modal activities postulated by Escher-Desrivières and Verrier (1951).

The more recent controversy over the possible function of rods in daylight vision is considerably preceded historically by that of the question of color vision in vertebrates. In fishes, the presence of color sensitivity was repeatedly disclaimed by von Hess (1912, 1922), one of the most prolific and distinguished contributors of his time (see discussions by von Frisch, 1912, 1913, 1924; Warner, 1931; Wunder, 1936; Walls, 1942). Von Hess' contention was based primarily on the lack of proper control of brightness perception in research concurrent with his, and the apparent demonstration in his own experiments of similar discrimination through brightness only. Von Hess, however, fell into the very pitfall by which he discredited others. His assumption that equal energy was transmitted by the spectral bands of a prism was at fault. Nor is it sufficient, as pointed out earlier, to use equal energy emission. For instance, Hineline (1927) in reporting on color vision in *Umbra limi* made this fundamental error. Preliminary experiments must be conducted to establish the spectral brightness sensitivity of the species under examination. Only when subjective brightness has been equated for different wavelengths can significant selective behavior be attributed to color discrimination. There must then be added the difficulties contributed by using normal behavior responses (Hamilton, 1922). In unconditioned fish the absence of a differential response when presented with a choice of two colors cannot be interpreted as the absence of the ability to detect a difference but may be merely the result of the absence of attaching any significance to the difference. Color in nature is usually associated with particular forms or patterns, which collectively make up environmental stimuli. Pre-conditioning by training to associate color and food has been employed in most cases. This method is not entirely free from objections since fatigue and adaptation can be involved, and, to the consternation of the experimenter, animals may become readily conditioned to the presence of unsuspected associated stimuli. Reeves (1919) has made one of the most thorough examinations of the problem, conducting discrimination tests on species of the genera *Semotilus*, *Lepomis*, *Pimephales*, *Notropis*, and *Umbra*. Using light sources which could be varied in intensity, minimum brightness discrimination for white light was first established in the dace (*Semotilus*) and sunfish (*Lepomis*). Subsequently significant selection between white and red light shone on ground-glass plates of matched brightness, followed by discrimination tests between red and blue at all intensities of red, established the presence of a color sense.

Both von Frisch (1912, 1913) and Mast (1915) experimenting with *Phoxinus* and *Paralichthys*, respectively, made use of adaptive responses to colored backgrounds as a means of demonstrating color sense. Response to intensity dominated but did not entirely account for the resulting patterns. Most of von Frisch's experiments have been based on conditioned responses (reviewed by Warner, 1931). By training *Phoxinus* to feed from test tubes lined with colored paper, accurate selection of the color from among a series of fifty grays ranging from white to black was recorded. When the colored tubes were compared directly (although brightness would not be controlled) blue, green and red were distinguished, whereas red and yellow were quite regularly confused.

The conclusion to the question of color vision may perhaps be summed up best by the words of Walls (1942) "No reasonable student of the problem any longer doubts that fishes–all duplex teleosts at least–can experience hue as a sensation-quality apart from brightness."

The almost complete absence of cones in the eyes of elasmobranchs would appear to preclude them from the possibility of having color vision.

### D. Phototransmission

Photochemical activity in the retina gives rise to nerve impulses in the optic tract, signaling the degree and condition of illumination. Although knowledge of electrical potentials associated with the eye dates from as early as 1849 (Raymond's discovery of "negative variation," as reported by Granit, 1947), it was not until the more recent development of electronic amplification that detailed studies of the rapid action potentials normally discharged from sensory endings were possible (Adrian, 1930). The intensity of the stimulus determines the *rate* rather than the *size* of discharge in each nerve fiber. Illumination of the eye generates a retinal action potential. The distal ends of the visual cells become negative with respect to the ganglion layer (Hartline, 1935). No comparable chain of neurones and synapses linking sensory endings to conducting nerve fibers is found in any other sense organ. Adrian and Matthews (1928) remarked that this arrangement in the optic pathway can hardly fail to affect the nature of the optic discharge. They studied discharges in the optic tract itself, by direct nerve stimulation, using the eel (*Conger conger*) as a test animal. The transmission characteristics were those of any sensory nerve, with the typical all-or-none response without any appreciable difference in time relations of action currents. Further experiments have involved tracing the excitations produced by illuminating the retina (Adrian and Matthews, 1927a, b, 1928). Either the eye and the tract were completely removed from pithed specimens or the eye was exposed but left *in situ* with circulation intact. Sudden illumination of an area of the retina produced a well-marked action current. Since the number of fibers in the eel's tract is about 10,000 the average area of retina supplied by each fiber is around 90 μ in diameter. Pin-points of light of 10μ diameter, however, activated more than one fiber illustrating the intercommunication within the retinal layers. The pattern of discharge at the start of steady illumination showed

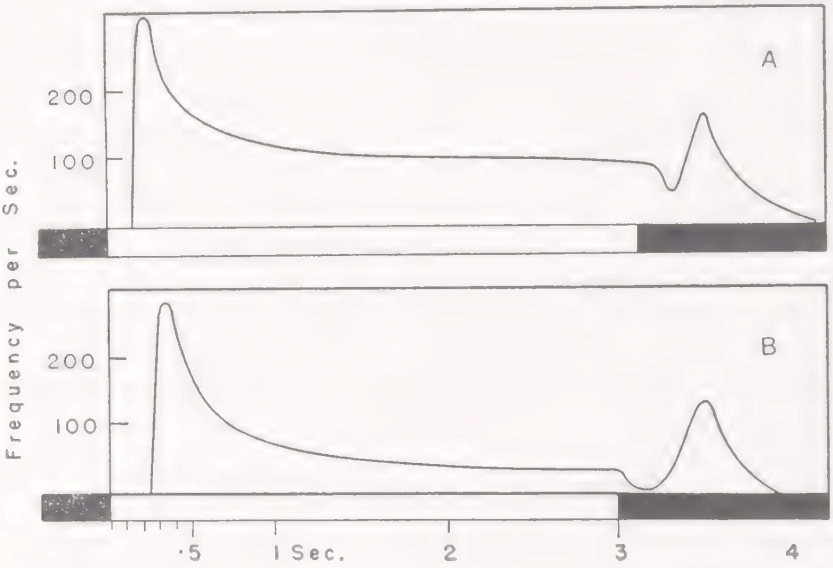

FIG. 8. Frequency of impulses per second in the optic tract of the eel (*Conger conger*), during and after retinal illumination. Duration of illumination (830 meter candles) shown by white strip below record. Area of illumination in (A) about eight times that in (B) (from Adrian and Matthews, 1927).

an initial latent period (0.1 seconds) followed by a rapid ascent to maximum discharge frequency (Fig. 8). This in turn was followed by a decline to a steady rate approximately one-quarter that of the maximum. When the light was switched off, there was a sudden increase in frequency and then a decline to zero ("off effect") signalling the change from light to darkness. By continuously moving the light-spot over the retina, maximum frequency is maintained but of somewhat irregular character. This is clearly of functional importance since moving objects must result in a greater sensory stimulus than stationary ones; it supports the view that the eel's eye is mainly an organ for the perception of movement. Propagation of the action potential along the optic tract of the catfish is more rapid in the periphery than the central core (Buser and Scherrer, 1950). Speeds of 14 m./second and 7 m./second, respectively, were recorded.

The process of conduction *through* the retina has many of the features of conduction in a reflex arc while the relations *within* the retina are more characteristic of those of the central nervous system with its network of dendrites and synapses.

More recent experiments (Adrian, 1946) suggest that the mammalian and avian response to a light flash can be divided into a rapid and slow component, the prominence of each component showing some relation to the wavelength. Although this is suggestive of a mechanism for transmission of color perception, the present state of knowledge is too limited for safe speculation. Hartline *et al.* (1952) emphasize that "it is not yet possible to trace, step by step, the physical and chemical events that intervene between the action of a stimulus on a receptor and the response of the associated afferent fibre."

In nature, we can picture the teleost visual elements constantly discharging in a pattern which symbolizes uniformity and which is, in effect, telling its possessor "nothing new" or "all is well." As the scattered light of early morning gradually penetrates the darkness of the water and illuminates the retina, there is a slow advance of the cones by contraction of the myoids in the direction of the infiltrating light. Synchronously the rods relax and become shielded by the pigment in the advancing retinal epithelial cells; some loss of sensitivity results but acuity is enhanced. Then, a sudden change in pattern of discharge with a localized increase from the peripheral area of the retina indicates the approach of a moving object. The rate of discharge increases; closer resolving is set up through a reflex chain; a particular light-dark-color pattern signals *predator*. The increasing retinal discharges dominate the sensory field and the fish central nervous system is alerted to further change of the retinal image. It moves again and the fish retreats maintaining the image pattern focused on its eye by increased tension of the retractor lentis. The shadow passes, the discharge rate diminishes, light illuminates the retina more evenly, the retractor relaxes—all is well again!

## IV. FUNCTIONAL ADAPTATIONS [4]

In general, the teleost eye is not specialized to the point of being either strictly nocturnal or diurnal but has remained serviceable throughout most of the day by virtue of a high proportion of rods to cones and an efficient photomechanical system. Elasmobranchs almost entirely lack cones and protective retinal pigments and are mostly suited to dim light. Highly contractile pupils save them from "over-exposure" when, like the basking shark, *Cetorhinus*, they lie close to the surface in full sunlight. Particular modifications occur, however, in those fishes which have taken on a specialized way of life.

### A. Deep-Sea Adaptations

In the bathypelagic zone, extending from about 200 to 2000 meters, many of the deep-sea fishes have remarkably large eyes despite the lack of visible sunlight (below, at most, 400 meters). *Bathylagus*, *Zenion*, and *Epigonus* possess relatively the largest eyes of any vertebrates. The faint glow of bioluminescence from fishes and from luminous invertebrates must provide the light source in the ocean depths. The fish eyes are characterized by enlarged pupil and lens, frequently a lack of epithelial pigment and numerous elongated rods (up to 25,000,000 per sq. mm. of retinal surface) linked in large groups to single bipolar cells; all these modifications can be related to efficient dim-light perception.

Increased ocular size is accompanied in some bathypelagic fish by a modification to a tubular eye shape (Fig. 9). An immense lens converges light on an inferior portion of the retina which has become relatively enlarged, the optic tract leaving from the medial edge. Accommodation is limited or absent. The upward aim of these tubular eyes suggests that they receive light from the ventrally cast glow of photophores of other fish, rather than that reflected from their own light organs.

### B. Aerial Adaptations

The optical problems of vision in air, while still retaining a functional aquatic eye, have been met by a number of fish in unique fashions. When exposed in air the cornea becomes a greater refracting surface than the lens; its curvature and surface optics are of paramount importance. An exceptional range of accommodation is required to overcome the greatly increased myopia. The unique, four-eyed top minnow of South and Central America, *Anableps anableps*, has a neatly divided eye which is raised above the surface and almost bisected by the waterline (Fig. 10A). The retina is modified and the lens is elongated in the dorsoventral axis and aquatic images are focused on a separate, superior region of the retina while aerial images are formed on an inferior retinal surface by refraction of light through the appropriately curved cornea and the flattened surface of the short axis of the lens. The result is a dual optical system, arranged to accommodate both aerial and aquatic vision simultaneously (Fig. 10B). Periodic immersion of the aerial portion prevents surface desiccation.

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[4] Taken largely from Walls (1942), otherwise direct reference is made.

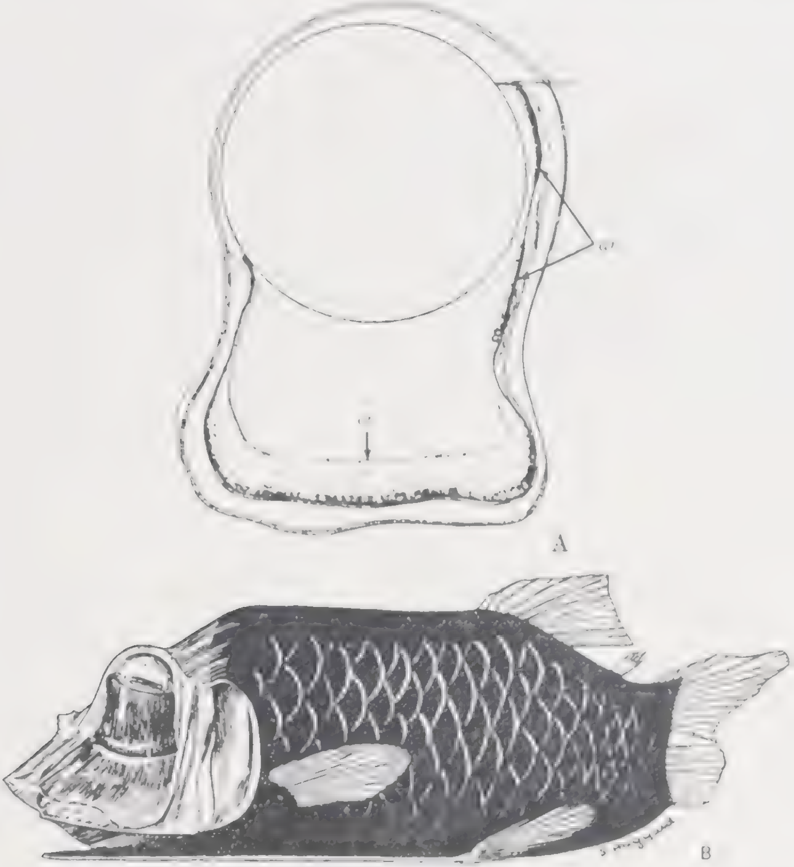

FIG. 9. Tubular eye of deep-sea fish. A: Diagrammatic vertical section, *Odontostomus hyalinus* × 13. B: *Opisthoproctus soleatus*. From Walls, 1942, after Brauer. ar, accessory retina; cr, chief retina.

In the mud skippers, *Periophthalmus* and *Boleophthalmus*, which spend much of their time on exposed tidal flats, turret-like, mobile eyes with strongly curved cornea and slightly flattened lens provide good retinal focus when maximally accommodated. These adaptations have gone so far that even complete relaxation of the retractor lentis probably does not restore precise vision under water. A high percentage of cones, reaching 100% in the inferior half of the retina, may be associated with the greatly increased brightness of aerial conditions.

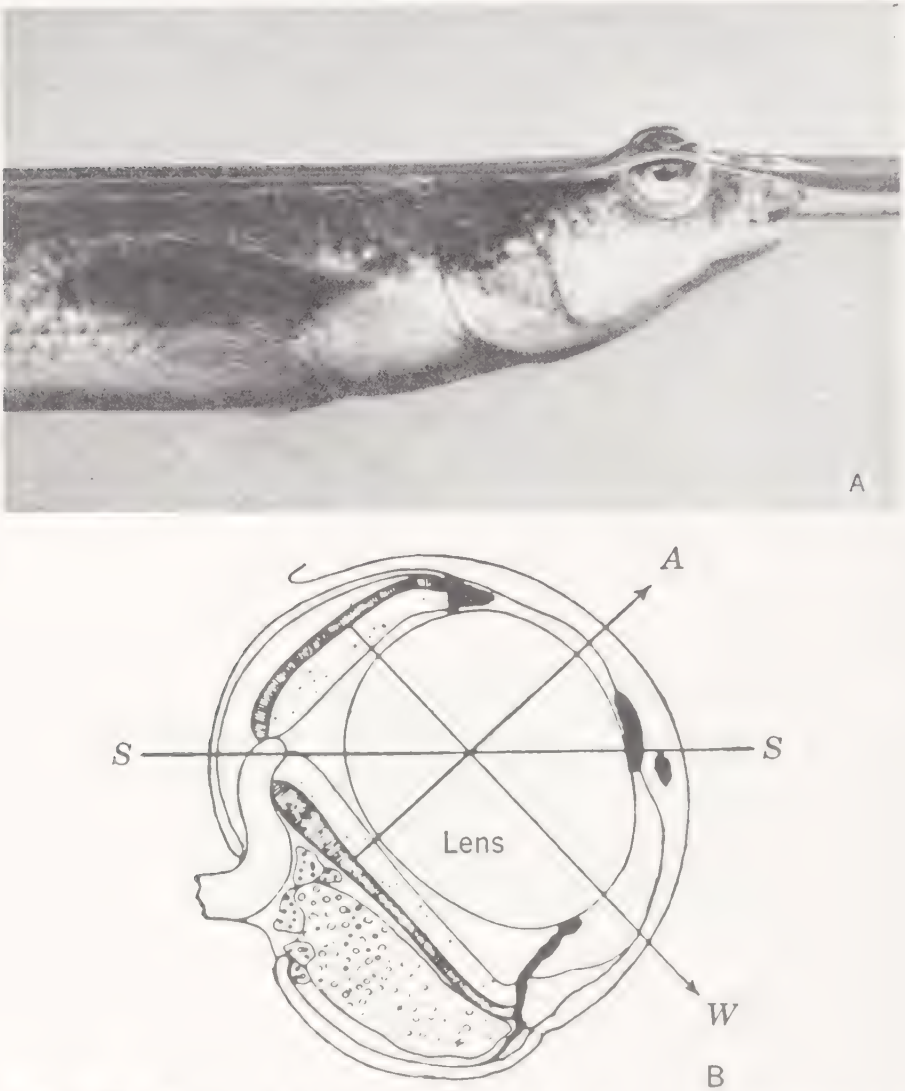

Fig. 10. The "four-eyed fish," *Anableps anableps*. A: Photograph showing aerial pupil above water when cruising at surface. B: Schematic vertical section (from Walls, 1942). S-S, plane of water surface; A, line of aerial sight; W, line of aquatic sight.

### C. Burrowing Adaptations

In some of the truly abyssal fishes and certain cave fishes, the eye is completely degenerate (Bridges, 1940; Schlagel and Breder, 1947), but burrowing fishes have retained their eyes on a reduced scale. Nayar (1951) has described four Indian species belonging to the families Symbranchidae and Gobioidae. These small, eel-like fishes display the burrowing habit during adverse conditions. One species, *Amphipnous fossorius*, has been found buried two to three feet in soft mud. The sunken eye is covered by a thick semi-transparent adipose skin formed from several layers of epidermal cells and a layer of connective tissue, leaving the surface flush with the surrounding skin. The relatively large and spherical lens is depressed almost in contact with the retina. Cones are either degenerate or lacking, and rods situated far apart. The lack of either a falciform process or hyaloid vessel indicates a low oxygen demand of the eye and an obviously reduced significance in the habits of these fish.

## VI. SYNOPSIS

There is a vast literature dealing with the vertebrate eye; much concerns the mammalian eye; the avian, reptilian, amphibian, or fish eye has been treated less frequently. Regarding one aspect of vision, wavelength discrimination, Pumphrey (1949) has commented that "the evidence available from animals other than mammals, though extremely relevant, is still scanty; inferences from structure and function cannot be relied on." Polyak (1941) critically reviewed concepts of the probable function of the retina among primates. Major objections were raised concerning insufficiency of knowledge of the retinal structures, the interaction of rods and cones in the perception of white light at different intensities, the reliability of the Duplicity Theory, the explanation of color sense and the structural insufficiency for explaining the degree and variation in retinal acuity. Such criticisms can be even better applied to other vertebrates and are a stimulus to further investigation.

The eye is not a simple structure. The dioptrics of the transparent media, the chemistry of the photosensitive pigment, the generation and transmission of the stimuli in the retina are all complex phenomena. It is not unnatural that some authors disagree and that gaps in knowledge are large.

The classes Osteichthyes and Chondrichthyes number over 20,000 living species. Free to move in a three-dimensional medium and venturing at times into the poorly supporting atmosphere, there has been great opportunity for the evolution of diverse patterns of eye. General-

## TABLE I

Synoptic Table of Eye Structure and Function in Teleosts and Elasmobranchs[a]

| Structure or Function | Teleost | Elasmobranch | Remarks |
|---|---|---|---|
| Cornea | Smooth; some irregular | Smooth + ? | Optical density almost same as water (1.33); highly refractive in air only |
| Iris | Immobile mostly (1) | Contractile | (1) Teleosts rarely with an "operculum"—contractile in eels, stargazers, flatfish (Young, 1931, 1933b) |
| Pupil | Large and round (1) | Some contract to a horizontal or vertical slit (2) | (2) Moderate rate of contraction (7 min.), slow dilatation (½ hr. +) |
| Lens | Spherical and inflexible | Spherical and inflexible | Flattened in some amphibious fish, e.g. *Periophthalmus* |
| Normal focus | Myopic (3) | Hypermetropic | (3) Not supported by some workers, e.g. Verrier (1948) |
| Accommodation | Lens retracted toward retina | Lens protracted away from retina | Considered by Verrier and associates to result from deformation of ocular globe |
| Tapetum of guanine | In choroid and retina (4) of marine fish | In choroid only | Conceals retina in transparent larval stage.<br>(4) Mainly deep-sea fish |
| Retina | Rods predominate over cones (5) | Almost pure rods (6) | (5) Deep-sea teleosts have pure rod retinas.<br>(6) *Mustelus* and *Myliobatis* have cones |
| Rhodopsin | Marine teleosts (7) | Exclusively | (7) Predominates in saltwater-spawning catadromous fish, e.g. eel |
| Porphyropsin | Freshwater teleosts (8) | Absent | (8) Predominates in freshwater-spawning anadromous fish, e.g. salmon |

| | | | |
|---|---|---|---|
| Scotopic vision | $\lambda_{max.} = 500–540$ mμ (blue-green) | $\lambda_{max.} = 505$ mμ (9) | (9) See Bayliss *et al.* (1936) |
| Photopic vision | $\lambda_{max.} = 590–610$ mμ (10) (orange) | Few or no cones | (10) For *Lepomis* (Grundfest, 1931), *Cyprinus* and *Tinca* (Granit, 1941) |
| Color vision | Present (11) | Absent | (11) Probably varies among species and with age (Warner, 1931) |
| Brightness transition (rods to cones) | 0.02–0.08 (12); 0.1–1.0 (13) millilamberts | Absent | (12) *Phoxinus* (von Buddenbrock, 1952).<br>(13) *Xiphophorus* (Crozier and Wolf, 1940) |
| Visual cells | 225,000 (14) to 56,200 (15) per mm² | 100,000 (16) to 21,600 (17) per mm² | Examples, (14) *Periophthalmus;* (15) *Esox;* (16) *Chimaera;* (17) *Torpedo* |
| Nerve fibers | 90,000 (14) to 3500 (15) per mm² | 5500 (16) to 600 (17) per mm² | (14) to (17) see Walls (1942); von Buddenbrock (1952) |
| Retinal pigment | Extensive in diurnal teleosts | No pigment (18) | (18) Shielded by contractile iris |

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[a] The numbers in parentheses in the second and third columns refer to the corresponding numbers in parentheses in the fourth column.

ization is hazardous but with this in mind the accompanying synoptic table (Table I) may be looked upon as a modal representation of structures and functions in the two major groups of living fish.

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## CHAPTER II: PART 2

# THE SENSE ORGANS: THE ACOUSTICO-LATERALIS SYSTEM

O. LOWENSTEIN

*Page*
I. Introduction . . . 155
II. The Lateral Line . . . 156
  A. Structure . . . 156
  B. Function . . . 158
    1. Conditioning and Elimination Experiments . . . 159
    2. Oscillographic Analysis . . . 160
III. The Ampullae of Lorenzini . . . 164
  A. Structure . . . 164
  B. Function . . . 166
IV. The Labyrinth . . . 168
  A. Structure . . . 168
  B. Function . . . 169
    1. Equilibrium . . . 169
      a. The semicircular canals . . . 170
      b. The otolith organs . . . 173
    2. Hearing . . . 178
      a. Frequency range and pitch discrimination . . . 179
      b. The localization of acoustic function . . . 181
  References . . . 182

## I. INTRODUCTION

Phylogenetically the acoustico-lateralis system of sense organs is as old as the vertebrates. In one form or other it is found in all living craniates, and in fossil fishes it can be traced back to the Silurian. The double-barreled name gives expression to the fact that the lateral line canals of the fishes and aquatic Amphibia, and the labyrinth of all craniates are considered closely related because of their ontogenetic origin from a well-defined system of adjoining placodes. There is also a striking uniformity in the morphology of their end-organs which consist of secondary sensory hair cells, the hair processes of which are ensheathed or embedded in more or less viscous gelatinous cupulae or in covering membranes. The latter are usually weighed by inclusions of concretions of lime crystals or foreign particles such as minute grains of sand or other inorganic detritus taken up from the environment.

Aberrant in form and function, but of similar ontogenetic origin, are the ampullae of Lorenzini of the elasmobranchs and holocephalous fishes, and probably related to them the vesicles of Savi in *Torpedo*.

## II. THE LATERAL LINE

### A. Structure

Fundamentally the end-organs of the lateral line system consist of groups of secondary sensory cells called neuromasts situated among the epithelial cells of the outer layer of the epidermis. In what are known as "free sensory hillocks" the hair processes of the sensory cells protrude above the ectodermal epithelium and are ensheathed in an elongated gelatinous cupula, waving freely in the surrounding water (Fig. 1). They are thus ideally suitable to transmit to the sensory hair processes the effect of water movements relative to the body surface. Such water movements past a stationary fish or arising during active locomotion, deform the cupula by deflecting it from its resting position. The longer and thinner the cupula, the more highly sensitive its response. Wave-like disturbances in the water radiating from objects moving in the vicinity of the fish, and the water pressure wave preceding an approaching object, have similar mechanical effects.

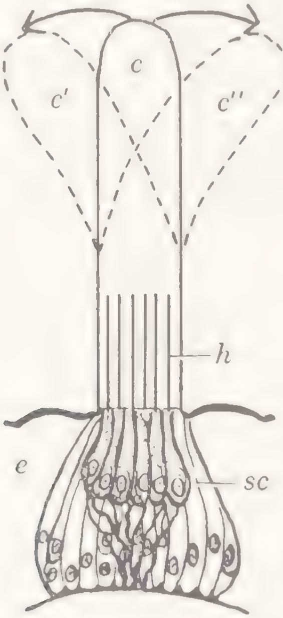

FIG. 1. Free sensory hillock of the lateral line system in a bony fish. c, c′, c″, cupula; e, epidermis; h, hair processes of sensory cells; sc, sensory cells and nerves (modified from Dijkgraaf, 1952).

The end-organs of the lateral line system are distributed over the body surface along lines, the pattern of which is remarkably constant in its fundamental topographic features: one line along the flanks of the body from head to tail, sometimes giving rise to secondary longitudinal lines, one shorter and less constant mid-dorsal line, and a system of lines on the head running external to branches of the VIIth, IXth, and Xth cranial nerves (Fig. 2).

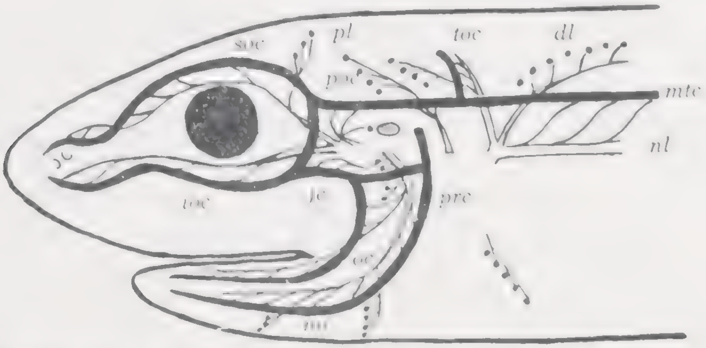

FIG. 2. Generalized distribution of lateral line organs and canals and their nerve supply. dl, dorsal line of pit organs; ioc, infra orbital canal; jc, jugal canal, mc, mandibular canal; mtc, main canal of trunk; nl, lateral line nerve; oc, oral canal; pl, anterior of three lines of pit organs; poc, postorbital canal; prc, preopercular or hyomandibular canal, soc, supraorbital canal, toc, transverse occipital canal (from Goodrich, 1930).

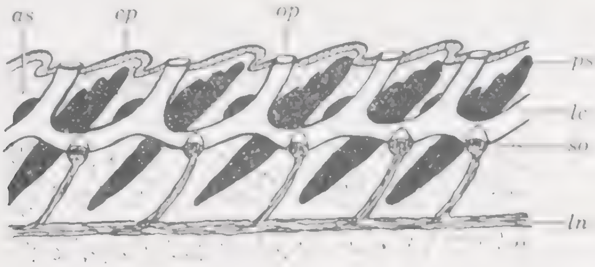

FIG. 3. Lateral line canal on the trunk of a bony fish (longitudinal section). as, arch of scale covering the canal, ep, epidermis; lc, lateral line canal, ln, lateral line nerve; op, external opening of canal; ps, posterior edge of scale, so, sense organ in canal (from Goodrich, 1930).

The delicate structure of the sensory hillocks, especially of their cupulae, makes it understandable that in many cases they are protected from mechanical damage or from over-stimulation by being sunk below the surface of the integument into pits, elongated furrows, and finally completely enclosed canals (Fig. 3). Free hillocks and canals can co-exist in one and the same individual, and there are interesting ecological relationships between habitat, mode of life, and the elaboration of the lateral line system (see p. 164). It is tempting to speculate on the phylogenetic events, and to assume an evolutionary sequence from hillocks to enclosed canals. However, the first fossil documentation of the lateralis organs is in the form of elaborate canal systems embedded in the superficial skeleton (Stensiö, 1926; White, 1935). A good account of the salient morphological facts can be found in Goodrich (1930).

### B. Function

Hofer (1908) was the first to show conclusively that the lateral line is a receptor for water currents. In his paper a good historical account can be found of the various earlier hypotheses concerning lateral line function. Hofer based his conclusions on the observation of the responses to stimulation with quantitatively controlled water jets directed against the various regions of the lateral line system in *Esox lucius*, combined with operative elimination of the lateral line organs by interruption of nerve supply and cauterization of the end-organs (Hofer, 1908; Wunder, 1927).

In elasmobranchs, a momentary change in the respiratory rate could be brought about by gently prodding the lateral line canals after the cutaneous tactile organs had been put out of action by destruction of the spinal cord in the region of stimulation. Water currents directed against such a region had the same effect (Parker, 1909).

Orientation with respect to the direction of water currents, is of vital importance, especially in fish living in streams and rivers, but also in marine fish inhabiting tidal waters. Fish keep their station in a stream for long periods of time by swimming against the current just fast enough to compensate for its speed. Changes in current speed and direction are promptly compensated by what are known as rheotactic responses, and the lateral line was for a time thought to be the sense organ chiefly concerned with the control of rheotactic behavior. That this is not so was shown fairly conclusively by Dijkgraaf (1933) who investigated the behavior of blind fish in linear currents of various cross-sections. Such fish exhibit very prompt rheotactic responses as long as they can find tactile contact with the wall or the bottom of the experimental tank. Slight frictional contacts of very short duration are sufficient to ensure perfect rheotactic responses. The performance of blind fish was not significantly impaired by the operative elimination of the lateral line, as long as the innervation of the tactile organs on the head was not affected. Dijkgraaf did not find any evidence of rheotactic orientation in the absence of both visual and tactile clues. In such a case fish move passively downstream. In a normal fish visual orientation by means of opto-motor responses and labyrinthine responses to passive rotary deviations from the direction of movement collaborate in the control of rheotactic orientation of the freely floating fish. In darkness tactile control appears to be the chief mechanism.

### 1. Conditioning and Elimination Experiments

This is one of the interesting cases where the combination of conditioning and operative elimination have proved of great value. Dijkgraaf (1933) worked on a number of mostly freshwater fish. Blind individuals learn fairly quickly to associate small water disturbances in their vicinity with the presence of either food or punishment and—what is more—they locate the center of disturbance with astonishing accuracy. Glass rods with discs of varying diameter at their end are spotted at a distance when being moved towards the fish. In the conditioning experiments, such a glass rod was immersed in the water immediately before and while food was offered. Conditioning is easily and quickly established, and characteristic food searching reactions are elicited by immersion of the glass rod which is accurately located and snapped at, especially when the mechanical disturbance coming from the slightly vibrating source is directed against the head or the tail. Stimulation of the flank induces the fish to turn its head in the direction of the mechanical disturbance during the search for food. A minnow (*Phoxinus phoxinus*) so conditioned accurately located a $^1/_4$ mm. diameter glass filament without terminal disc vibrating with an amplitude of 2 mm. at a distance of 10 mm. from head or trunk.

Small diameter water currents flowing from pipette nozzles under quantitatively controlled pressures produced responses similar to those caused by moving solid objects. Most of the fish investigated showed similarly good responses with the exception of *Ameiurus nebulosus* in which thresholds for mechanical stimuli were higher. It was suggested that this might be due to a natural preponderance of chemical orientation in this fish, causing inhibition of the response to mechanical stimulation unaccompanied by an associated chemical stimulus.

*Corvina nigra* and *Acerina cernua* have exceptionally large head canals, and localization of water disturbances is correspondingly acute.

Operative elimination of the whole system of lateral line organs without accessory damage to the tactile sense endings in the integument of the head region is not easy. The organs of the trunk are denervated by section of the lateralis branch of the vagus below the pectoral girdle followed by its complete extraction. The head organs offer the chief difficulty. Joint section of the trigeminal and facial branches near the orbit combined with cauterization of all organs or removal of the integument in the temporal region, was adopted by von Frisch and Stetter (1932) and Dijkgraaf (1933). The obvious disadvantage lies in the interference with the function of the tactile endings in the skin which are supplied by the trigeminal fibers. In the minnow Dijkgraaf succeeded in cutting the dorsal branch of the facial nerve alone, near the labyrinth. This rather delicate operation was employed only in experiments in which a differential analysis of tactile and lateral line function was aimed at.

All the above described conditioned responses fail after such elimination of the organs of the lateralis system. Responses to small diameter currents are also affected by damage to lateralis organs, whereas orientation in large diameter straight and curved currents is unaffected both in seeing and in blind fishes by the total bilateral elimination of the lateralis organs.

Whether sound vibrations represent an appropriate stimulus has been investigated by a number of authors, obviously in view of the fact that the lateral line had primarily been considered to be an accessory auditory organ, an interpretation still widely adhered to by physiologists. Von Frisch and Stetter (1932) studied the auditory responses in labyrinthectomized minnows with and without a functionally intact lateral line system, and found that the remnant of low frequency sound responses, after the elimination of the acoustic parts of the labyrinth, could be explained solely as a function of the tactile skin sensitivity.

It is also interesting that locomotor activity is not basically affected by interference with the lateral line organs. It has been postulated that these organs may function as accessory proprioceptors signaling the magnitude and progression of body flexion during swimming (Hofer, 1908; Steinmann, 1914; Hoagland, 1935; Sand, 1937). The only effect on locomotion to be observed in blind fishes after total elimination of the lateral line organs is a certain "reluctance" to move, more especially in the early post-operative period. Frequent collisions with the aquarium walls and with solid obstacles occur, but there is no detectable lack of muscle coordination affecting the swimming movements themselves.

### 2. Oscillographic Analysis

The question of what constitutes the adequate biological stimuli for the lateral line sense organs has been fairly satisfactorily and plausibly answered by the combined training and elimination experiments reviewed above. The physiological mechanisms underlying their mode of function can, however, be best studied by oscillographic analysis of the stimulus–response relationships of the end-organs. Such experiments have been carried out by Hoagland (1933a–d), Schriever (1935), Sand (1937), and Katsuki *et al.* (1950, 1951, 1952).

Hoagland, working on the catfish (*Ameiurus*) and the trouts (*Salvelinus* and *Salmo*), recorded the action potentials from filaments of the lateralis branch of the vagus, and discovered that the end-organs are spontaneously active with a resting discharge of impulses. Mechanical stimulation by stroking or pressing on the skin above the lateral line, by weak water currents directed against it, and by passive flexing of the trunk, modified the impulse discharge picture. Tuning forks of 100–250 cycles per second held against the side of the vessel containing the preparation also affected the discharge picture. Such stimuli produced no absolute synchronization, although a rhythm of 20–70 beats per second was imposed upon the resting discharge. Hoagland also found that the discharge frequency from the lateralis endings increased exponentially with temperature.

Sand (1937), working on the elasmobranch *Raja*, found it possible to study the responses of single end-organs to a quantitatively controlled perfusion of the lateral line canals. Cannulae can be inserted into the end pores, and a closed perfusion system established. The oscillographic records were taken from preparations of the isolated pectoral fin containing the hyomandibular loop. Such preparations show a vigorous and complex resting discharge, and Hoagland's observations could be fully corroborated with respect to the various types of stimuli used by him. Perfusion of the canal evoked a strong over-all increase in the discharge activity, and adaptation was very slow even when perfusion extended over a period of 60 seconds. Multi-fiber preparations responded equally to perfusion in opposite directions. In preparations with a few functional units only, there appeared, however, a clear difference in the effects of head- and tailward perfusions. Two groups of receptors could thus be isolated. Group one responded to headward perfusion with a strong increase in discharge frequency, whereas tailward perfusion inhibited the discharge activity. A silent period at the end of headward and an accelerated rate of discharge at the end of tailward perfusion characterized this group. The second group behaved *mutatis mutandis* in exactly the opposite way.

The behavior of a single receptor is illustrated by Fig. 4. This shows that one and the same receptor is fundamentally capable of signaling both perfusion directions by opposite changes in its resting activity which can be either increased or decreased by oppositely directed stimuli. The discovery of this type of behavior had previously been made in work on the end-organs of the semicircular canal in the dogfish labyrinth (Lowenstein and Sand, 1936), when its highly significant importance in connection with the bi-directionality of this receptor organ was pointed out by the authors (see below). The behavior of single lateralis units thus brings them into line with those of the labyrinth, and there arises the question whether in the lateral line the bi-directionality of the spontaneously firing sense endings is of specific functional significance, or whether it is just a by-product of spontaneity.

Not all endings are spontaneously active, and it was found that "spontaneously silent" units are made to discharge during perfusion in one and at the end of perfusion in the opposite direction. An analysis of the quantitative stimulus–response relationship showed a logarithmic relation over a range of flow rates between 2 mm. per second and 16 mm. per second. No threshold could be determined, as clear responses were obtained with the slowest rates of movement obtainable under the conditions of the experiments.

So far as the interpretation of the over-all picture is concerned, Sand came to the conclusion that the two antagonistic receptor types in the lateral line are mixed in a random assortment within each group of sensory cells, and no special significance was attributed to their orientation.

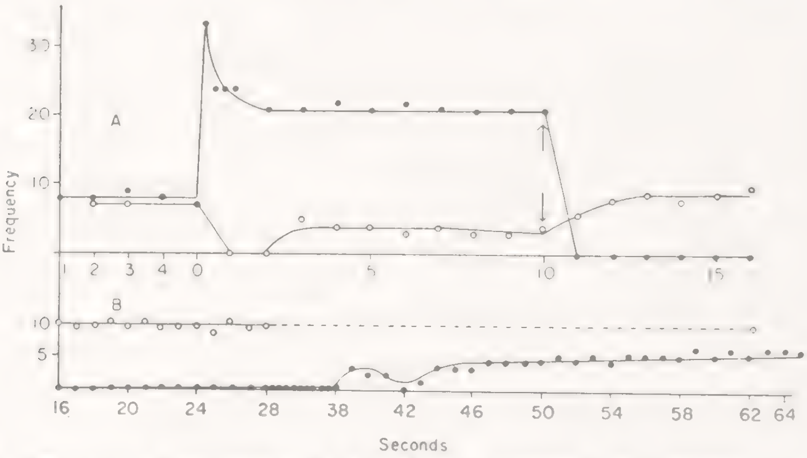

FIG. 4. Response frequencies of a single end-organ of the hyomandibular canal of the ray (*Raja clavata*) in perfusions at 6 cm. water pressure (rate of flow 12 mm./sec.). Dots, headward perfusion; circles, tailward perfusion. Perfusions begin at zero time and end at arrow. B is the continuation of A (from Sand 1937).

The physiologically operative stimulus was shown to be the actual movement of fluid in the canal and not its pressure.

Working on the Japanese eel, *Anguilla japonica*, and on the "sea eel," *Rhynchocymba nystromi*, Katsuki *et al.* (1950, 1951, 1952) claimed to have obtained clear evidence for the presence in the lateralis end-organs of two different types of receptor endings supplied by thin and thick nerve fibers, respectively. They found that the fiber diameter varied continuously from 4 to 15 $\mu$, the majority measuring 10 $\mu$. The organs innervated by thin fibers generally yielded a pronounced spontaneous discharge of impulses; they adapted slowly and had only moderate maximum discharge frequencies (40 impulses per second). The organs connected to thick fibers had no resting discharge, adapted rapidly, and had a higher threshold and maximum discharge frequency. Stimulating the lateral line preparations by flowing water, mechanical pressure, and touch, and by linearly rising galvanic currents, the authors came to the conclusion that pressure changes are the physiologically operative stimulus. They failed to find organs, the electrical activity of which could be inhibited by peripheral stimulation, but they confirmed the existence of two different kinds of fibers activated by oppositely directed stimuli. When low-frequency, vibratory stimuli were applied to the lateral line preparation, the thin and thick fibers differed in the frequency range with which they could fire in synchrony. Thin fibers responded synchronously up to 10 cycles per second, whereas the thickest fibers showed a discharge of 1 impulse per cycle up to a frequency of 100 cycles per second. The authors suggest that these results provide evidence in favor of a participation of the lateral line in sound perception, as postulated by Hoagland (1935).

Finally, it may be mentioned that the possibility of the lateral line organs functioning as temperature receptors has also been envisaged. Following Hoagland's (1933b) report of an exponential temperature effect on the spontaneous discharge frequency in the lateralis organs, Rubin (1935) claimed to have found that partial elimination of the lateral line innervation in catfish *Ameiurus*, sunfish *Eupomotis* and *Lepomis*, perch *Perca flavescens*, and others, interfered with what he considered to be specific responses to increase in temperature. Dijkgraaf (1940), combining again the method of conditioning with separate elimination of the cutaneous and lateralis receptors, convincingly demonstrated that on the one hand the lateral line receptors are not at all capable of producing temperature responses in trained fish after elimination of their spinal cutaneous sense endings, and on the other hand that the elimination of the lateral line does not in the least interfere with the accuracy of temperature responses in fish with intact cutaneous receptors.

To sum up then, the lateral line organs in fishes are chiefly concerned with what Dijkgraaf calls "distant touch" orientation (Ferntastsinn). In an aquatic environment where optic orientation is of reduced accuracy, these organs are capable of supplementing vision by helping to localize objects at a distance. These objects may either be moving (prey, enemies) and thus constitute the focal point of a mechanical disturbance, or their presence and localization may be perceived and accurately computed from the time relations of reflected water waves set up by the swimming movements of the fish itself. The latter case would fundamentally conform with the principle of "echo-location." A fairly accurate three-dimensional sensory representation of the topographic features of the immediate environment, as well as the localization of moving objects in the vicinity, may thus be assumed to be mediated by this sense organ in the absence of visual orientation either in darkness or in a turbid medium.

Insofar as local currents can influence the orientation of a free-swimming fish, the lateral line must, however, be considered as an accessory rheotactic organ of subordinate importance. The localization of the in-flow of a small trickle of a stream into a pond may be a case in question. The recognition of eddy currents and the consequent localization of obstacles and general topographic features in the immediate vicinity of the fish represents an interesting case on the border line with distant touch perception.

It appears unlikely that the lateral line participates to any significant extent in hearing, and the same holds with regard to its reputed role as a temperature receptor.

Dijkgraaf quite rightly points out that the electrophysiological results in fact only delineate the range of "physiologically adequate" stimuli which are not all necessarily utilized by the animal in the elaboration of its orientation reactions, i.e. they are not to be considered as "biologically adequate."

The development of free sensory hillocks and canal systems appears to be related in an interesting manner to the mode of life and habitat of the fish. In preponderantly free-swimming forms, in which the body is continuously exposed to the action of relative water movement, the canal system is usually well developed, thus furnishing protection against wear and tear of the delicate cupula formations. In bottom-living fish (*Misgurnus fossilis* L.) and inert fish like the pike (*Esox lucius*), canal systems are found to be secondarily reduced. On the other hand, canal systems persist even in bottom-living fish if they inhabit rapid streams (*Nemacheilus barbatula*) and the surf zone of the sea shore.

## III. THE AMPULLAE OF LORENZINI

### A. Structure

The ampullae of Lorenzini form a striking system of jelly-filled canals which open all over the rostral region and the rest of the head of the elasmobranchs. In addition, they are found in one type of tropical marine siluroid (*Plotozus anguillaris* Bl.) (Friedrich-Freska, 1930). They are ontogenetically and perhaps also phylogenetically related to the acoustico-lateralis system, although in their minute structure and very likely also in their function they have very little in common with it. They were discovered by Malpighi and first described in 1663, but the first detailed description was in 1678 by Lorenzini whose name they bear to the present day. At first they were considered to be mucus-secreting glandular organs, but later their sensory nature became recognized and they were thought to be functionally associated with the lateral line organs. A short historical review of the study of the ampullae can be found in a paper by Dotterweich (1932). Figure 5 shows the distribution of the sensory canals and their openings in the elasmobranch *Acanthias vulgaris* as described by Metcalf (1915). They are exclusively innervated by branches of the facial nerve. In their simplest form the sensory canals consist of a relatively long duct filled with a jelly-like substance which does not give a specific mucus reaction. The duct takes its origin from an ampulla-like basal part which in most cases is subdivided into a system of secondary ampullae (Fig. 5). Simple undivided ampullae are found in *Torpedo* and in the siluroid *Plotozus*. The canals can be of considerable length and can reach a diameter of up to 4 mm. Dotterweich studied the minute innervation of the ampullary epithelium, and came to the conclusion that both constituent cell types, namely the so-called pyramid cells (hitherto considered to be supporting cells) and the flask cells (hitherto described as typical sensory cells) are innervated. He described the nerve process of the pyramid cells as being continuous with the cell body. No such nerve processes, however, are described by Friedrich-Freska in what he calls the supporting cells separating the flask cells of the *Plotozus* ampullae. The latter, according to this author,

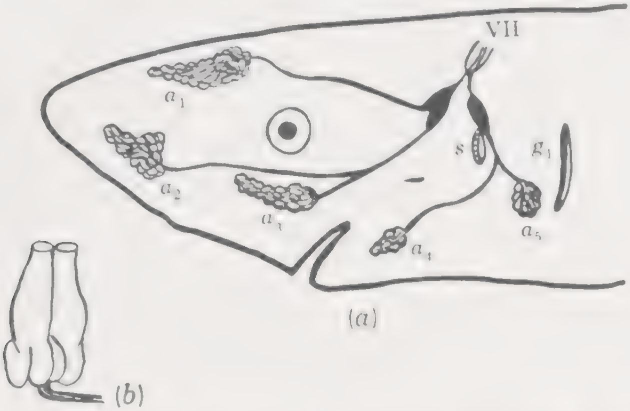

FIG. 5. (*a*): Distribution of ampullae of Lorenzini in a dogfish ($a_{1-5}$). VII, roots of facial nerve; $g_1$, first gill slit; s, spiracle. (*b*) Shape of ampulla in *Squalus acanthias*. Magnification: × 10 approximately. From Dotterweich, 1932.

are typical secondary sensory cells. Dotterweich, on the other hand, believes that the flask cells are purely secretory, and ascribes the receptor function to the pyramidal cells which would have to be classified as primary sensory cells. We are here clearly confronted with a dilemma.

### B. Function

Matters are still further complicated by the fact that the two chief methods of investigation into sensory function, namely the elimination experiment and the oscillographic study of stimulus–response relationships, have led to entirely different conclusions. Reasoning that the ampullae appeared to be particularly suited to function as receptors for changes in hydrostatic pressure, with the canal jelly becoming less viscous under higher pressure, Dotterweich carried out the following experiments in support of his hypothesis. He injected distilled water under the integument in the vicinity of the canal organs in the dogfish *Scyliorhinus*, and compared the behavior of individuals with intact ampullary organs and those in which the nerve supply to the ampullae had been interrupted before injection. After a latent period during which the distilled water presumably penetrated into the canals and led to a swelling of their gelatinous contents, the fish began to swim snout upwards as if they were seeking to rise to the surface from greater depths. This attitude was maintained after reaching the surface, and they continued to swim about like this for one or two hours. Fish with interrupted nerve supply, however, never showed this type of behavior. Viscosity measurements in a centrifuge showed that there is an inverse relationship between centrifugal force and viscosity, which suggests the possibility of greater liquefication of the jelly under the influence of rising hydrostatic pressure. The sense organ would in this case serve to gauge the viscosity, and Dotterweich suggests that the sensory cells respond to variations in the intensity of Brownian movement. Changes in depth from surface to 3600 meters were claimed to be well within the discriminative range of the ampullary sense organs.

However, when Sand (1938) studied the impulse responses from the ampullary organs of the elasmobranch *Raja*, he failed to find any mechanical sensitivity whatsoever, in contrast to the neighboring lateralis fibers. When he recorded the impulse discharges from an ampullary nerve, he found a massive spontaneous discharge, the volume of which could not be influenced by touch, stroking, or pressure. Individual impulse trains in smaller nerve filaments were similarly unaffected by mechanical stimuli. When, however, cooled or warmed elasmobranch Ringer solution was dropped on to the skin overlying the mandibular ampullae, a highly sensitive response to temperature change was discovered which paradoxically consisted of an increased discharge rate on cooling, and a drop in the discharge frequency on warming. The discovery of this temperature response was followed up by quantitative experiments on single end-organs of the mandibular ampullae of the ray. Figure 6 shows the impulse rates of single sensory units in response to temperature changes of less than 0.5° C., and the paradoxical increase in discharge rate on cooling and corresponding decrease on warming can be clearly seen. This effect can be observed over a wide range of temperatures, namely 5–20° C. Under conditions of stable equilibrium, the discharge frequencies after adaptation do in fact vary as a function of temperature, displaying a positive temperature coefficient, and thus behaving like other biological systems. In discussing his results, Sand compared these temperature effects with what had been found in lateral line organs and stretch receptors, and he stressed the important fact that the latter organs too show the paradoxical temperature effect, but with an entirely different order of magnitude, with a temperature change of 10° C. producing only a comparatively insignificant response. It thus appears that the paradoxical temperature effect is probably common to a number of different sensory end-organs, but its exaggerated "efficiency" in the Lorenzini ampullae makes it probable that it may here form the basis for a true temperature receptor mechanism. It is worth mentioning in this connection that in *Xenopus laevis*, which possesses a persisting system of lateral line organs, the lateralis endings also display a paradoxical temperature effect which is intermediate in its sensi-

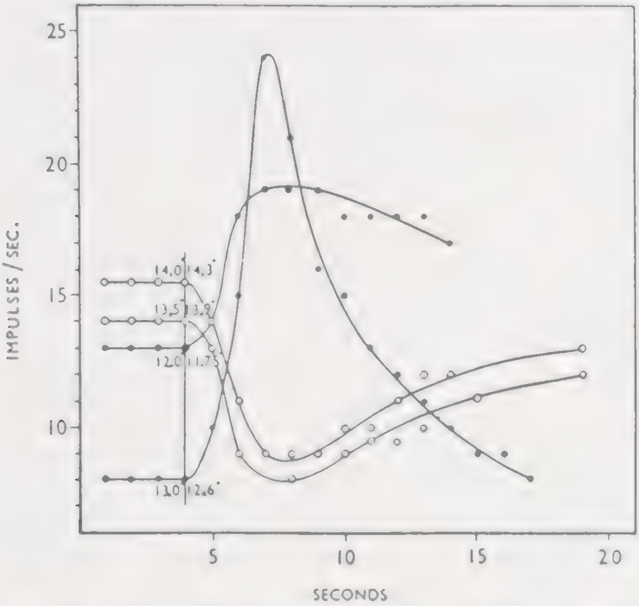

FIG. 6. Responses of single units of a mandibular ampulla of Lorenzini to temperature changes of less than 0.5° C. Dots, response to cooling, circles, response to warming (from Sand, 1938).

tivity between that of the elasmobranch lateral line organs and of the Lorenzini ampullae (Murray, 1956).

If we then accept the suggestion of Sand that the Lorenzini ampullae function as highly sensitive receptors for temperature changes, it still remains for us to understand the significance of their structural peculiarities, especially of the long jelly-filled canals opening by pores distributed in fields all over the head integument. Sand offers no suggestion apart from pointing out that in electrophysiological experiments the temperature response mechanism works in isolated end ampullae in the absence of the canals. However, the relation between the arrangement of the organ groups and the direction of the respiratory current is striking. In the ray the superficial ophthalmic, the inner and outer buccal, and the mandibular and hyomandibular groups all lie on surfaces bathed by the respiratory water.

No cutaneous nerve endings with a sufficiently acute temperature response could be found in the ray, and Sand therefore came to the conclusion that the Lorenzini ampullae are in fact the only temperature receptors that these animals possess.[*]

## IV. THE LABYRINTH

### A. Structure

The labyrinth has been considered to represent the final stage in the process of submergence of neuromast organs of the lateral line type by having become completely or at any rate almost completely separated from the outside medium, and enclosed in the brain case. The ductus endolymphaticus maintains a tenuous connection with the outside world in the elasmobranchs only. The labyrinth originates ontogenetically from a common placode with the lateral line. Its typical subdivision into semicircular canals and otolith organs is phylogenetically fundamental, and is found in the labyrinth of the Cyclostomata and, according to Stensiö (1927), in the fossil Ostracoderm *Kiaeraspis*. Figure 7 shows the two chief morphological types of labyrinths occurring in the fishes, and apart from the absence of the horizontal semicircular canal in the cyclostomes and the persistence of an external opening of the ductus endolymphaticus in the elasmobranchs, the chief variation concerns the relative proportions and positioning of the otolith organs, utriculus, sacculus, and lagena. The complete absence of a cochlea or any cochlear rudiment differentiates the fish labyrinth from the inner ear of all other vertebrates.

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[*] In a recent publication (Murray, 1957) electrophysiological evidence is presented according to which the ampullae may, after all, have a specific mechanoreceptive function, serving in the detection of local changes of water pressure near the surface of the body. They differ from the lateralis organs by their slower or less complete adaptation, and would therefore react to slower and longer lasting pressure stimuli

## B. Function

The labyrinth has four chief functions; it is an organ which is: (1) concerned with the maintenance and regulation of muscle tone; (2) a receptor for angular accelerations; (3) a gravity receptor; (4) a sound receptor. This follows from the fact that bilateral total labyrinth extirpation is followed by temporary or lasting loss in muscle tone, by severe disturbances of equilibrium, and by deafness.

### 1. EQUILIBRIUM

In the fishes the effector organs showing equilibrium reflexes are the eyes, the fins, and to a lesser degree, the trunk musculature. The reflexes are controlled by the receptors of the semicircular canals and of the otolith organs, and occur as compensatory rotary and vertical eye movements and postures during active and passive changes of position from the "normal." Rotation of the fish in a horizontal plane evokes horizontal rostro-caudal eye deviations and nystagmus, easily observable when watching an aquarium fish executing right and left turns. These reflex movements and postures of the eyes facilitate the fixation of a certain visual field during spatial displacement of the head. When the compensatory eye deviation has reached its anatomical maximum, the eye is brought back with a jerk to its normal position in the orbit. Compensatory deviation and jerk-like return together are known as the nystagmus representing its slow and quick phase, respectively. The slow phase runs counter to and the quick phase in the same direction as the rotary displacement of the head. The slow phase is controlled by the labyrinth

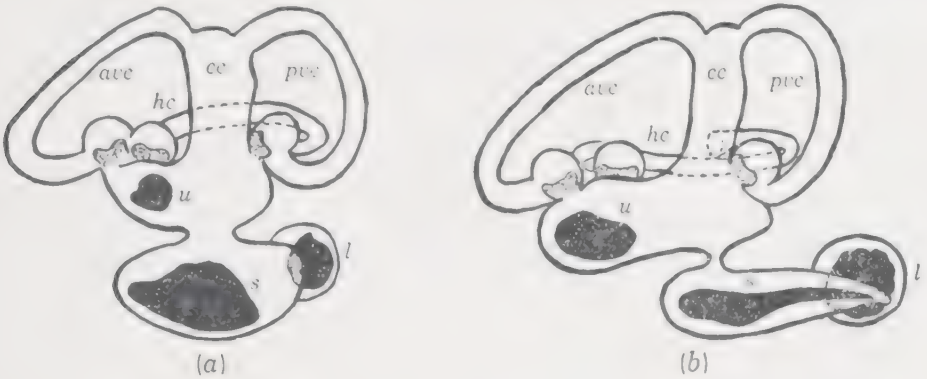

FIG. 7. The two chief types of labyrinths in fish. (*a*): "Normal" type (trout); (*b*): ostariophysian type (minnow). (Right labyrinths, median aspect.) avc, anterior vertical canal; cc, crus commune, hc, horizontal canal, l, lagena with asteriscus; pvc, posterior vertical canal; s, sacculus with sagitta; u, utriculus with lapillus (modified from von Frisch, 1936.)

(and optokinetically by the eye itself), whereas the quick phase is of central nervous origin.

Figure 8 shows the reflex response of the fins of the minnow, *Phoxinus phoxinus,* to passive sideways tilting towards the right. The posture of the fins automatically leads to a righting of the fish as soon as a forward movement takes place (righting reflex) (Lowenstein, 1932). Reflex bending of the trunk to right and left occurs in response to left and right turns, respectively.

*a. The semicircular canals.* The semicircular canals are fluid-filled canals which widen at one end into an ampulla and make open connection at the opposite end with the vestibular cavity. The ampullae, too, open into the vestibulum so that there is complete continuity of the endolymph system. The receptor structure of the canals is found in the ampullae in the form of a sensory crista with a neuro-epithelium composed of supporting cells and of neuromast cells, the hairs of which are ensheathed in a gelatinous cupula terminalis. Since in life the cupula has about the same refractive index as the endolymph and shrinks drastically on fixation, its size was grossly underestimated until Steinhausen (1931, 1933, 1935) showed that it extends to the opposite wall of the ampullary dome, and during its deflections glides in swing-door fashion along it with a minimum of endolymph leakage. It was thus established that cupula and endolymph form a rigidly coupled system, and that the elastic cupula is to be defined as a highly damped torsion pendulum with a period, in the case of the pike (*Esox lucius*) in the neighborhood of 20 seconds. Deflections of the cupula under the impact of angular accelerations in the appropriate plane of space represent the stimulus to which the animal reacts by dynamic effector reflexes in the form of compensatory movements of eyes and limbs. In the elucidation of the mode of function of the semicircular canals as such, and the interaction of the six canals in the maintenance of dynamic equilibrium, elimination experiments and experiments with artificial mechanical and caloric stimulation have served to establish a fair amount of informa-

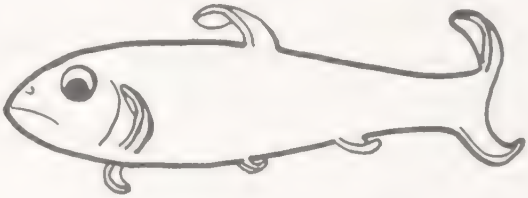

FIG. 8. Compensatory fin posture and vertical eye deviation in a fish tilted on to its right side (from Lowenstein, 1936).

tion (Maxwell, 1923; Lowenstein, 1936). There remained, however, a hard core of controversial problems, the solution of which had to await the results of oscillographic analysis of the responses from isolated labyrinthine end-organs. Fortunately, the elasmobranch labyrinth was found to be eminently suitable for this type of experiment. The various branches of the VIIIth nerve are relatively easily accessible through the cartilaginous skull, and, what is more, the organ survives for considerable periods of time in the isolated brain case. A thorough qualitative and quantitative analysis of the stimulus–response relationships governing the function of the semicircular canals, could thus be carried out in the labyrinth of the dogfish (Lowenstein and Sand, 1936) and the ray (*Raja clavata*) (Lowenstein and Sand, 1940a, b; Groen, Lowenstein, and Vendrik, 1952; Lowenstein, 1954). It was found that there is a spontaneous discharge of sensory impulses from a considerable propor-

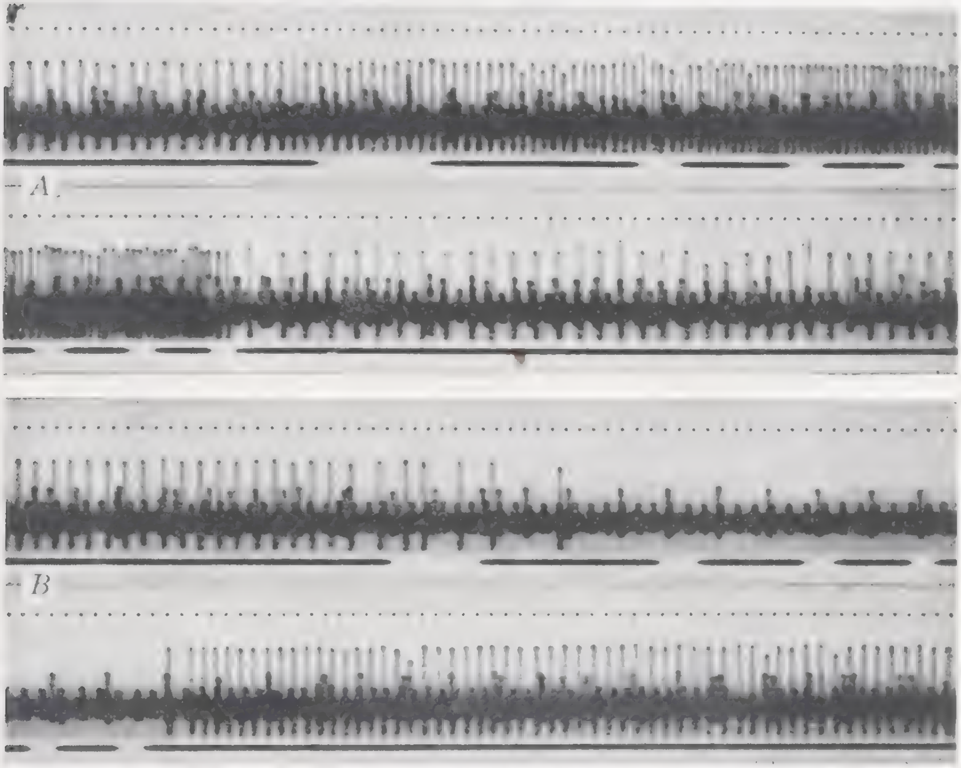

FIG. 9. Impulse discharge from a single end-organ in the left horizontal semicircular canal of the ray (*Raja clavata*) in response to acceleration to the left (A) and to the right (B). The lower strip of each part is the continuation of the upper. Time at the top of records is 0.05 second. Acceleration is recorded by the signal line at the bottom. Successive gaps mark the revolution of the turn-table through 12° (from Lowenstein and Sand, 1940b).

tion of the sense endings from each ampulla, when the labyrinth is at rest (resting discharge). During angular displacement in the appropriate direction the discharge frequency of impulses is increased or inhibited as the case may be (Fig. 9). The horizontal canal responds to rotation about the vertical primary axis of the fish, but is unaffected by rotation about its longitudinal and transverse axes. The impulse discharge is augmented by ipsilateral (ampulla-trailing) and inhibited by a contralateral (ampulla-leading) rotary displacement.

The anterior and posterior vertical canals respond to rotation about all three primary axes. During rotation about the horizontal longitudinal axis, the four vertical canals function in pairs with anterior and posterior canals of one side being arrayed against those opposite, and the discharge activity is augmented in the canals undergoing ampulla-leading and inhibited in those undergoing ampulla-trailing displacement.

Post-rotatory after effects in the form of post-stimulatory depression and post-inhibitory augmentation of the discharge activity are clearly correlated with well-known effector responses, such as the post-rotatory eye nystagmus.

A functional correlation between the discharge activity in the six semicircular canals and the reflex responses of the six eye muscles was established, and fulfilled satisfactorily all aspects of Sherringtonian muscle antagonism with respect to the labyrinthine eye reflexes (Lowenstein and Sand, 1940a).

The existence of a spontaneous discharge in the end-organs of the semicircular canals is functionally highly significant. Above all it serves here as the background for bi-directionality, i.e. for the sensitivity of a given semicircular canal to rotations in opposite direction which lead to an increase or decrease in the impulse discharge rates. Apart from this, the steady influx of spontaneous activity from the sense organ into the central nervous system must contribute to the maintenance of muscle tonus. This was directly demonstrated in the pike (*Esox lucius*), where operative interruption of the nerve supply to one horizontal ampulla led to a protracted asymmetry in the tonus of the horizontal eye muscles. This asymmetry could be augmented by ipsilateral and diminished by contralateral horizontal rotation, a fact furnishing further evidence for the basic bi-directionality of the semicircular canal mechanism (Lowenstein, 1937).

An interesting structure which has been interpreted as a functional substitute for the missing horizontal semicircular canal in *Petromyzon* is a system of two large endolymphatic cavities lined with ciliated epithelium. Two counterrunning endolymph vortices are constantly maintained in each cavity by vigorous ciliary activity. The ensuing endolymph movements do not, however, appear to spread to other parts of the labyrinth, although the ciliary cavities are in open communication both with the ampullae and with the space containing the maculae (de Burlet and Versteegh, 1930). In the absence of any additional sensory endorgans specially related to the ciliary sacs, de Burlet and Versteegh declined to elaborate a hypothesis about their function. Such a hypothesis has recently been formulated by Mygind (1948), who assumes that the endolymph vortices can be gyroscopically affected by movements of the animal in a horizontal plane. When thus made to deviate from their usual direction, they are held to bring about pressure changes in the neighboring vertical ampullae in such a way that motoric compensatory reactions are elicited. Thus the ciliary sacs are said to act as a substitute for the horizontal semicircular canals wanting in these animals. It is difficult to judge the merits of this hypothesis. The fact that *Petromyzon* does show reflex reactions to angular accelerations about the dorso-ventral axis in the absence of horizontal canals does not in itself necessitate the assumption of an additional receptor mechanism, as it has been shown that vertical canals can be directly stimulated by horizontal rotations (Lowenstein and Sand, 1940a).

*b. The otolith organs.* The prototype for the otolith organ is fully elaborated in the invertebrate statocyst, and gravity perception is one of the fundamental factors in animal orientation. It is interesting, however, that an auditory function was originally attributed to all so-called otocysts. Eimer (1878) and Romanes (1885) were the first to apply the newly discovered equilibrium function of the vertebrate ear to the lithocysts of medusae, attributing to them, in addition to their assumed auditory function, the regulation of locomotory movements.

In the vertebrates the detailed study of the mode and function of the otolith organs has all along been handicapped by their inaccessibility to separate elimination or stimulation. The operative interruption of the nerve supply of individual otolith organs is generally almost impossible because of the shortness of the nerve branches, and the removal of the otoliths usually involves wide openings in the vestibular space with considerable endolymph loss and spread of the damage to all other parts of the labyrinth. It is therefore not surprising that until recently the functional analysis of the otolith organs was mainly based on the interpretation of the spatial arrangement and the anatomic relationships of the otoliths and their sensory epithelia, supplemented by rather contradictory results of experiments with artificial mechanical and other stimulations carried out in elasmobranchs by Kubo (1906) and Maxwell (1923), and in the pike by Ulrich (1935).

When it became clear, however, that fish react to sound over a considerable range of frequencies (see below), and that their power of sound perception and pitch discrimination is chiefly localized in the labyrinth, the conclusion became inescapable that otolith organs must be capable of gravity and sound perception alike. Apart from the fact that both sensory functions imply sensitivity to linear acceleration, they are so different in their biological significance that it was hard to believe that any single one of the three otolith organs, namely utriculus, sacculus, and lagena, could combine both functions. The results of the search for a fundamental division of labor among the three otolith organs in fishes can be summarized as follows. In the elasmobranch *Mustelus canis* (Parker, 1909; Maxwell, 1923), in the bony fish *Pseudopleuronectes americanus* (Lyon, 1899), and in *Carassius auratus* (Manning, 1924), *Cynoscion regalis* (Parker, 1908), and *Gobius jozo* (Werner, 1929), and finally in *Phoxinus phoxinus* (Lowenstein, 1932; von Frisch and Stetter, 1932), separate functional elimination of parts of the otolith system showed that the utriculus is able to control the whole range of postural responses to positional changes. These responses consist of lasting eye deviations and fin postures, the compensatory significance of which is the same as that of the dynamic effector responses to angular acceleration. Bilateral elimination of the sacculus and lagena complex leaves the fish with its gravity responses practically unimpaired, whereas the bilateral elimination of the utriculus abolishes the whole range of known postural reflexes. The anatomical subdivision of the labyrinth into a pars superior (utriculus and semicircular canals) and a pars inferior (sacculus and lagena) is therefore physiologically significant. Often the separation of these two parts is considerable, e.g. in the minnow (*Phoxinus*), only a narrow canal, the canalis utriculo-saccularis, joins them, while in *Gobius jozo* the two parts are completely separated. Corresponding results were obtained in all other vertebrate classes (Lowenstein, 1936), although there remained a suspicion that in some cases the lagena may participate in the control of equilibrium [Schoen and von Holst (1950) in the fish, *Gymnocymbus ternetzii;* MacNaughton and McNally (1946) in the frog].

The extension of the oscillographic analysis of impulse responses from the semicircular canals to the less accessible otolith organs carried out by Lowenstein and Roberts (1950) in the elasmobranch *Raja clavata* showed quite clearly that the utriculus macula does in fact respond to positional changes in all directions, and that it is thus potentially capable of controlling the whole range of postural responses. Figure 10 shows a single-unit response of the utriculus to a full circle tilt about the horizontal longitudinal axis (lateral tilt). The discharge frequency shows a clear maximum near a position in which the labyrinth under observation lies uppermost (side up), and a minimum near the spatially opposite position (side down). A similar picture would be obtained during fore-and-aft tilting about the horizontal transverse axis of the fish. Besides units which have static discharge rates strictly corresponding with certain spatial positions of the labyrinth (position receptors), there are

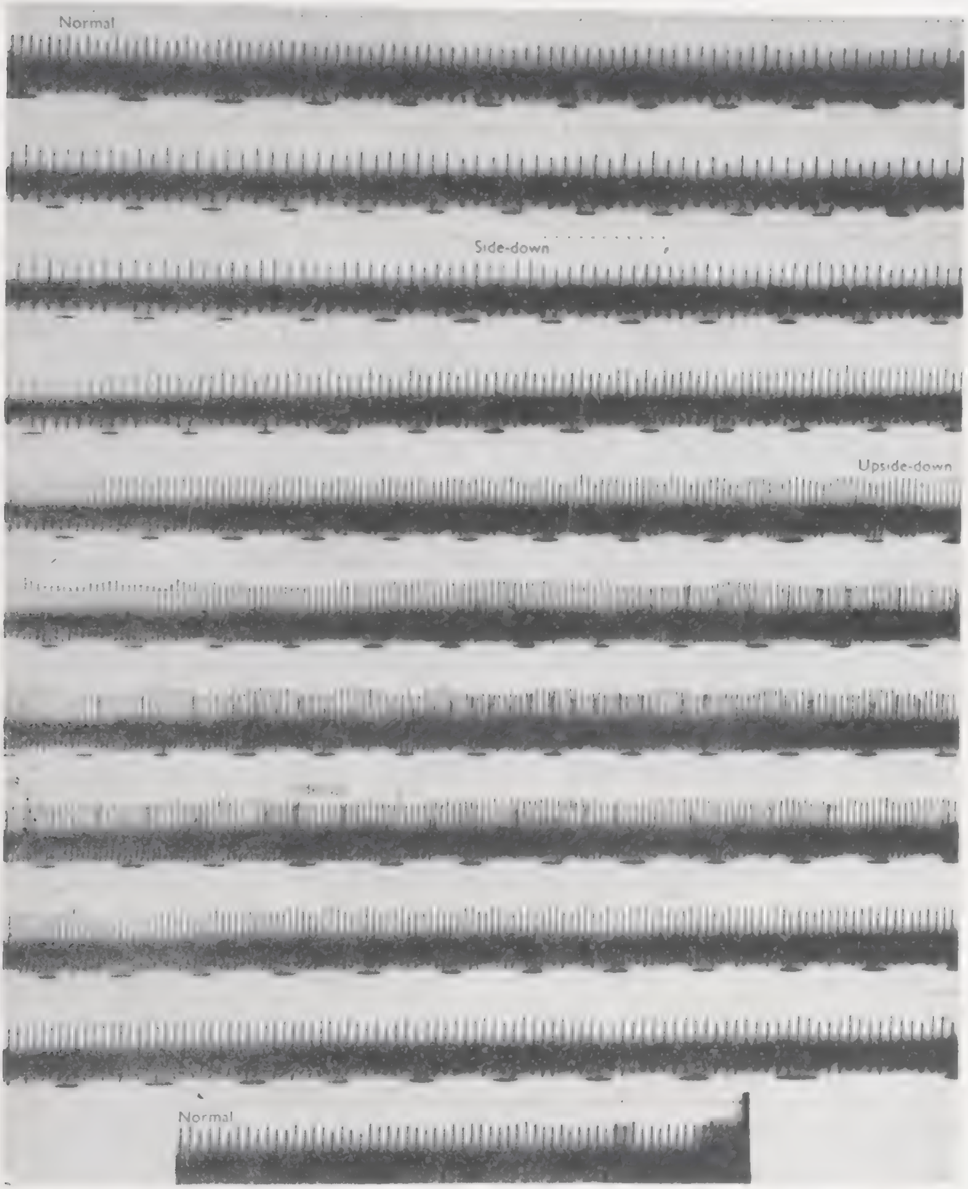

FIG. 10. Continuous record of the response from two end-organs of the utriculus of the ray (*Raja clavata*) to a full-circle lateral tilt. Time marker at top of record 24/second. Rotation signal at the bottom: 1 gap/3°. Constant speed of tilting 10°/second approximately. The maximum discharge frequency lies near the side-up and the minimum near the side-down position (from Lowenstein and Roberts, 1950).

TABLE I

A LIST OF TELEOSTS IN WHICH HEARING HAS BEEN RELIABLY DEMONSTRATED, WITH DATA FOR FREQUENCY RANGES WHERE AVAILABLE. NO SIMILARLY RELIABLE DEMONSTRATION OF HEARING IS AVAILABLE FOR NON-TELEOSTEAN FISHES[a]

| Family | Species | Frequency range (cycles per second) Low | High | Reference |
|---|---|---|---|---|
| NON-OSTARIOPHYSI | | | | |
| Anabantidae | *Anabas scandens* (= *A. testudineus*) | | over 659 | Diesselhorst (1938) |
| | *Betta splendens* | | 2637–4699 | Schneider (1941) |
| | *Colisa lalia* | | | Schneider (1941) |
| | *Macropodus cupanus* | | | Schneider (1941) |
| | *Macropodus opercularis* | | | Schneider (1941) |
| | *Trichogaster leeri* | | | Schneider (1941) |
| | *Trichogaster trichopterus* | | | Schneider (1941) |
| Anguillidae | *Anguilla anguilla* | | | Bull (1928) |
| | *Anguilla anguilla* | 36 | 488–650 | Diesselhorst (1938) |
| Cottidae | *Cottus gobio* | | | Stetter (1929) |
| | *Cottus scorpius* | | | Froloff (1925) |
| Cyprinodontidae | *Fundulus heteroclitus* | | | Parker (1904) |
| | *Lebistes reticulatus* | | | Farkas (1935) |
| | *Lebistes reticulatus* | 44 | 1200–2068 | Farkas (1936) |
| Embiotocidae | *Cymatogaster aggregatus* | | | Moorhouse (1933) |
| Esocidae | *Umbra limi* | | | Westerfield (1921) |
| | *Umbra pygmaea* | | | von Frisch and Stetter (1932) |
| Gadidae | *Gadus aeglefinus* | | | Froloff (1925) |
| | *Gadus callarias* | | | Froloff (1925) |
| Gobiidae | *Gobius niger* | | 800 | Dijkgraaf (1952) |
| | *Gobius paganellus* | | 600–800 | Dijkgraaf (1950) |
| | *Periophthalmus koelreuteri* | | up to 651 | Diesselhorst (1938) |
| Labridae | *Crenilabrus griseus* | | | Froloff (1925) |
| | *Crenilabrus melops* | | | Bull (1928) |
| | *Crenilabrus pavo* | | | Froloff (1925) |
| Mormyridae | *Gnathonemus sp.* | | 2794–3136 | Stipetic (1939) |
| | *Marcusenius isodori* | | 2069–3100 | Diesselhorst (1938) |

| | | | | |
|---|---|---|---|---|
| Percidae | *Acerina cernua* | | | Froloff (1925) |
| | *Perca fluviatilis* | | | Froloff (1925) |
| Sciaenidae | *Corvina nigra* | | 1024 | Dijkgraaf (1950) |
| | *Corvina nigra* | | 1000 | Dijkgraaf (1952) |
| | *Corvina nigra* | | | Froloff (1925) |
| Sparidae | *Sargus annularis* | | 1250 | Dijkgraaf (1952) |
| OSTARIOPHYSI | | | | |
| Characinidae | *Hemigrammus caudovittatus* | | | von Boutteville (1935) |
| | *Hyphessobrycon flammeus* | | 6960 | von Boutteville (1935) |
| | *Pyrrhulina rachoviana* | | | von Boutteville (1935) |
| Cobitidae | *Nemacheilus barbatula* | | 1740–3480 | Stetter (1929) |
| Cyprinidae | *Alburnus lucidus* (=*A. alburnus*) | | | Zenneck (1903) |
| | *Carassius auratus* | | | Bigelow (1904); Manning (1924); Denker (1931) |
| | *Carassius auratus* | | 3480 | Stetter (1929) |
| | *Carassius carassius* | | | Froloff (1925) |
| | *Idus melanotus* (= *Leuciscus idus*) | | | Denker (1931) |
| | *Idus melanotus* (= *Leuciscus idus*) | | 5524 | Stetter (1929) |
| | *Leuciscus dobula* | | | Zenneck (1903) |
| | *Rutilus rutilus* | | | Zenneck (1903) |
| | *Phoxinus phoxinus* | 25 | 5000–7000 | von Frisch (1938) |
| | *Phoxinus phoxinus* | 16 | 5000–6000 | von Frisch and Stetter (1932) |
| | *Phoxinus phoxinus* | | | Stetter (1929); Denker (1931); Benjamins (1934); von Boutteville (1935); Haden (1935) |
| | *Pimephales notatus* | | | McDonald (1921) |
| | *Tinca tinca* | | | Froloff (1925, 1928) |
| Gymnotidae | *Electrophorus electricus* | | 870–1035 | von Boutteville (1935) |
| Siluridae | *Ameiurus nebulosus* | | over 1300 | von Frisch (1938) |
| | *Ameiurus nebulosus* | | | Maier (1909); Haempel (1911); Parker and van Heusen (1917); Krausse (1918); von Frisch (1923); Stetter (1929) |

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[a] From von Frisch (1936) and Kleerekoper and Chagnon (1954).

other end-organs in the macula which react by a change in their discharge rate to the change of position as such, irrespective of the direction of the change, returning to a basic discharge rate whenever the head has come to rest in a new position (out-of-position receptors).

It was found that the posterior third of the sacculus macula closely adjoining the lagena also responds to positional changes, and that it does so in much the same way as the utriculus. The two structures therefore overlap in range, which means that one of them could be considered to be dispensable from the point of view of equilibrium control. The lagena, on the other hand, shows a distinctly different mode of reaction, responding to both lateral and fore-and-aft tilting, but showing a sharp maximum of the impulse discharge rate in or near the "normal" position. The lagena may therefore be considered to signal by a steep increase in activity the return of the head to the normal spatial position. The term into-level receptor used for the lagena by MacNaughton and McNally (1946) appears thus to be very appropriate.

Thus it appears that in the elasmobranch labyrinth sensitivity to gravitational stimuli is found in all three otolith organs. How far this is utilized in the elicitation of equilibrium responses is another question which cannot be decided by electrophysiological experiments. We shall return to the discussion of this question when dealing with the auditory function of the fish labyrinth.

Equilibrium in fishes is not controlled by the labyrinth alone. In the absence of highly developed proprioceptor mechanisms, visual orientation takes a considerable share in the control of posture and movement. This is supplemented by so-called dorsal light reactions which manifest themselves in a tendency of the animal to turn its dorsal side in the direction of the incidence of illumination. The collaboration of eye and labyrinth in this field has recently been analyzed by von Holst and his collaborators by means of most ingenious experimental methods (von Holst, 1950). These investigations have made a significant contribution to the understanding of quantitative and qualitative aspects of otolith function.

### 2. HEARING

The history of the problem of hearing in fishes has been reviewed in detail by von Frisch (1936) and by Kleerekoper and Chagnon (1954). It may suffice to recall here that from antiquity onwards fish have been believed to be endowed with sound perception. This was believed to be localized in the internal ear and also in the lateral line. However, under the impact of the discovery of the equilibrium function of the labyrinth in the first half of the 19th century, and in view of the absence in the fish labyrinth of any structure corresponding with the cochlea of the higher vertebrates, the assumption gained ground that fish may after all be deaf, and that all earlier evidence for their power of sound perception ought to be considered with suspicion as it was probably based on a misinterpretation of circumstantial evidence. Even if sound perception could be convincingly demonstrated in fishes, it was argued, the term "hearing" should be used only if it could definitely be shown that the inner ear was the chief receptor organ concerned. After the publication of work by Zenneck (1903), Piper (1906), and Parker (1904, 1909), evidence in favor of hearing in fishes began to accumulate. A number of workers based their claims on observations of direct responses of various species of fish to sound stimuli. Occurrence and repeatability of such responses was, however, often doubtfully documented, and no convincing picture emerged, chiefly because the various acoustic stimuli used were without biological significance for the experimental animal. Here, as so often, the method of conditioning helped to overcome this difficulty, and yielded convincing evidence in favor of sound perception over a considerable range of frequencies in a number of families of fishes.

*a. Frequency range and pitch discrimination.* Table I surveys the reliable data including, in a number of cases, the frequency range of sound perception.

The Ostariophysi show a significantly better performance so far as threshold, range, and pitch discrimination are concerned and this fact is held to be connected with the presence of a swimbladder and its link-up with the labyrinth by the chain of Weberian ossicles (von Frisch, 1936; Poggendorf, 1952). The evidence in favor of sound perception among teleosts is thus impressive, and makes the assumption of a well-defined biological significance of hearing inescapable. Sound production exists in a great variety of types of fish, and suggests the existence of acoustic communication among individuals. This aspect, however, lies outside the scope of the present chapter (but see Chap. IV).

Sound perception usually goes hand-in-hand with pitch discrimination. Table II summarizes the results in this field.

Successful experimental analysis of sound perception, and especially of pitch discrimination, presupposes successful application of the conditioning technique. It can therefore not be carried out in forms like the elasmobranchs in which conditioned responses cannot easily be established.

Two different types of pitch discrimination have to be distinguished, namely absolute discrimination between two frequencies when offered singly on different occasions, and relative discrimination of frequencies

### TABLE II
### TONE DISTINCTION IN VARIOUS SPECIES[a]

| Species | Range of Tone Distinction (cycles per second) | Reference |
|---|---|---|
| NON-OSTARIOPHYSI | | |
| *Umbra* sp. | 288 and 426 | Westerfield (1921) |
| *Anguilla anguilla* | Less than one octave[b] | Diesselhorst (1938) |
| *Gobius niger* | ¾ up to 1¼ tone[b]<br>(9–15% frequency distinction) | Dijkgraaf (1952) |
| *Corvina nigra* | ¾ up to 1¼ tone[b]<br>(9–15% frequency distinction) | Dijkgraaf (1952) |
| *Sargus annularis* | ¾ up to 1¼ tone[b]<br>(9–15% frequency distinction) | Dijkgraaf (1952) |
| *Marcusenius isodori*<br>(Mormyridae) | ¼ Octave[b] | Diesselhorst (1938) |
| *Gnathonemus sp.*<br>(Mormyridae) | One tone[b] | Stipetic (1939) |
| *Macropodus opercularis*<br>(Anabantidae) | Varying between one tone and 1⅓ octaves[b] | Schneider (1941) |
| OSTARIOPHYSI | | |
| *Phoxinus phoxinus* | A major third in 821–651 range; a minor third in 290–345 range | Stetter (1929) |
| *Phoxinus phoxinus* | ½ tone in 987.7–1046.5 range<br>(6% frequency distinction) | Wohlfahrt (1939) |
| *Phoxinus phoxinus* | ¼ tone in 400–800 range | Dijkgraaf and Verheijen (1949) |

[a] From Kleerekoper and Chagnon (1954).

[b] The frequency range in which distinction of tones was examined was not mentioned by these authors.

of equal intensity offered in rapid succession in the form of a warbling note (Wohlfahrt, 1939, 1950). In the latter case, discrimination reaches the astonishing lower limit of a quarter tone in the 400–800 cycles per second range. The absence in the fish labyrinth of a special sound analyzer structure of the basilar membrane type adds additional physiological interest to these findings.

So far as threshold sensitivities are concerned, very few reliable data are available. Poggendorf (1952) measured the absolute values of the threshold sound pressures in dynes cm.$^{-2}$ in *Ameiurus nebulosus* for a range of frequencies, and found a steep rise of thresholds above 1600 cycles per second. At the optimum frequency of 800 cycles per second, the absolute threshold is as low as that for the optimum frequency of 3200 cycles per second in man. At low frequencies *Ameiurus* is considerably more, and at high frequencies considerably less sensitive than man. In cyprinoids, too (Stetter, 1929; von Boutteville, 1935), the relative sensitivity to sounds in the middle frequency range was found to be of the same order of magnitude as in man.

Hearing without the power of fairly accurate localization of the source of sound would appear to be of rather restricted value to an animal. However, experiments on *Phoxinus* by von Frisch and Dijkgraaf (1935), and on *Phoxinus* and *Ameiurus* by Reinhardt (1935), both under natural conditions and in the laboratory, showed that only relatively high intensity sound offered at close range (10 and 20 cm.) had a directive effect, and this effect was not impaired by the operative elimination of the labyrinth, the Weberian apparatus, and the lateral line organs. The authors therefore attributed this limited sound localization to the tactile sensitivity of the skin. Kleerekoper and Chagnon (1954), using low frequency vibrations in relatively small tanks, observed that *Semotilus atromaculatus atromaculatus* (Mitchill) orientated towards lines of highest intensity in a complex pattern of standing waves and intensity gradients from one or more sources. The authors make no statement as to the nature of the receptor organs involved in these reactions.

*b. The localization of acoustic function.* There is no reason to doubt that in fishes, as in other vertebrates, the inner ear is the chief organ concerned with hearing, and the work of von Frisch and his pupils has yielded convincing evidence that, at least in the Ostariophysi, the pars inferior of the labyrinth, viz., the sacculus–lagena complex, is chiefly responsible for sound perception, whereas the equilibrium function of the labyrinth resides in the pars superior, viz., utriculus and semicircular canals. The fact that the sound-conducting structures, such as the Weberian apparatus and so-called acoustic windows in the cranium (von Frisch, 1938; Dijkgraaf, 1950), appear to be closely associated with the pars inferior in the Ostariophysi makes this assumption still more cogent. In other fish, such as the Clupeidae, the morphological situation is different. Here the swimbladder–labyrinth connection clearly aims at the utriculus, and the question arises (de Burlet, 1935; Wohlfahrt, 1936) whether potentially all otolith organs could take on an acoustic function, the decisive factor being the exposure to or insulation from vibratory stimuli, differing from case to case in accord with the morphological situation. That this may in fact be so is strongly suggested by the results of the electrophysiological experiments on the labyrinth of the elasmobranch *Raja clavata* (Lowenstein and Roberts, 1951). In these experiments propagated impulse discharges in response to low-frequency vibratory stimulation (up to 120 cycles per second) were recorded from nerve twigs from the anterior part of the sacculus macula, from the unloaded part of the utriculus macula (lacinia utriculi), and from the macula neglecta situated in the roof of the sacculus cavity. No such vibration responses were found in the lagena which proved to contain gravity receptors only. A cranial window, the fenestra of Scarpa, is anatomically related to the vicinity of the sacculus and the macula neglecta, and it is quite possible that the vibration sensitivity of part of the utriculus macula may not be utilized by the animal. Unfortunately, little is known about hearing in elasmobranchs, and no combined conditioning and elimination experiments appear to be possible with these fish.

Accepting the effect of a basic vibration sensitivity of the otolith organs in fishes, the great differences in the acuity of hearing and the power of pitch discrimination found to exist between the Ostariophysi and the rest may well be entirely due to the elaboration of resonators and sound conductors associated with the labyrinth. The remarkable fact of a highly developed power of pitch discrimination in the absence of a morphologically obvious frequency analyzer still remains one of the unsolved problems of fish physiology. It is probable, however, that this may be based upon a synchronization between the stimulus frequency and the frequency of the impulse discharge. The fact that pitch discrimination is absent above 800 cycles per second would be compatible with this assumption on neurological grounds (Lowenstein and Roberts, 1951).

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### CHAPTER II: PART 3

# THE SENSE ORGANS: OLFACTORY AND GUSTATORY SENSES OF FISHES

ARTHUR D. HASLER

*Page*
I. Introduction . . . . . . . . . . . . . . . . . . . . 187
II. Definition and Theory . . . . . . . . . . . . . . . 187
III. Anatomy of the Olfactory Organs of Fishes . . . . . . . . . 188
IV. Impulses from the Olfactory Tissue to the Brain . . . . . . . . 193
V. Taste and the Common Chemical Sense . . . . . . . . . . 195
VI. Olfaction in Fishes . . . . . . . . . . . . . . . . 198
  A. Perception of Food Odors . . . . . . . . . . . . . 198
  B. Recognition of "Body" Scents . . . . . . . . . . . . 199
  C. Alarm Substances . . . . . . . . . . . . . . . . 200
  D. Repellents . . . . . . . . . . . . . . . . . . 201
  E. Discrimination of Odors of Aquatic Plants . . . . . . . . 202
  F. Discrimination of Pure Chemicals . . . . . . . . . . . 203
VII. Orientation to the Parent Stream as It Relates to Odor Recognition . 204
VIII. Concluding Comment . . . . . . . . . . . . . . . . 206
  References . . . . . . . . . . . . . . . . . . . 207

## I. INTRODUCTION

As is true of so many aspects of structure and function in the fishes, the olfactory mechanism occurs in a multiplicity of variations, for here is a group of animals whose span of existence in geological time is so extensive as to have permitted endless opportunities for adaptation to many types of aquatic environments with their associated biotic and community odors. In addition, mutations favoring olfactory acuities have had great periods in which their survival value could be tested.

## II. DEFINITIONS AND THEORY

Odor perception in fishes will be interpreted here as those sensations chemically induced, which are relayed to the central nervous system from the olfactory sac. Taste, and what is termed the common chemical sense, will be referred to as sensations perceived by the gustatory nerve endings in the mouth and over the entire body in some species.

A smellable substance must pass into solution on the mucous film to be perceived by a terrestrial vertebrate; therefore, the view most generally accepted is that olfaction in all animals is aquatic in the final sense and this will be acknowledged here with no further comment.

We are concerned here with a sense of such refined acuity that it defies comparable attainment by the most sensitive instruments of modern chemical analysis. Man can detect the odor of methyl mercaptan at an approximate concentration of $9 \times 10^{-14}$ *M*, which is 10,000 times more dilute than the minimum concentration of an active taste stimulant such as strychnine hydrochloride. Moncrieff (1944) comments: "Man is microsomatic, yet how wonderful his sense of smell which detects quantities far beyond the limits of balances, microscopes, or other laboratory paraphernalia, with the possible exception of the spectroscope." In insects, only a few molecules in a liter must be present in the turbulent air being carried from a female moth to attract a mate from two miles distance. Odor thresholds for dogs are not known accurately. Certainly, the sense of smell is most acute in hunting breeds; and the ability to perceive the sex odor is notoriously keen.

One modern theory of odor perception (Kistiakowsky, 1950) suggests that odoriferous substances act by interfering with enzyme-catalyzed reactions in the receptors. Since enzymes are affected in their action by minute amounts of a variety of substances, this theory plausibly explains the high sensitivity of human sense of smell and the wide range of compounds that possess odors. The rapid reversibility of inhibitory effects on enzymes would account for the speedy adaptation of the reception system enabling it to register new odor impressions.

Classifications of odorous substances have been anthropomorphic for the most part (Moncrieff, 1944). What may be odorous to man may not necessarily be so for fish. Therefore, at this stage in our knowledge, little relation exists between types of sensation inducing physiological or behavioristic activity and the chemical nature of the stimulant.

Odor perception is often referred to as a distance perceptor, as in the case of emanations of a chemical in which a gradient of concentration exists to its source. Not only is its acuity greater than taste perception, but this sense is responsive to multifold substances, most of which are organic in nature.

In fishes, we are dealing with an acuity of olfaction which matches any attainment of terrestrial animals.

## III. ANATOMY OF THE OLFACTORY ORGANS IN FISHES

Among the fishes, extreme variations can be found in the morphology of olfactory organs. In the sharks and rays the paired olfactory pits are usually situated on the ventral side of the snout. The single opening of each pit is more or less divided by a fold of skin into an anterior inlet and a posterior outlet, the latter sometimes leading into the mouth. As the fish swims through the water, and particularly as it takes water into its mouth in breathing, a current of water is passed through each of its olfactory sacs. In this way the olfactory organs are influenced by the respiratory current. In the lungfishes the anterior apertures are external, whereas the posterior openings lie within the mouth and correspond to the choanae of higher vertebrates.

In *Salmo salar*, Gawrilenko (1910) described the embryological development beginning with the 24-segment stage when there is a paired thickening of the ectoderm immediately anterior to the eye forming an olfactory plate. By the 45-segment stage, this separates from the brain and the neural crest grows out to join the plate. When the embryos are 6–8 mm. long, the plate forms a bud which invaginates and becomes a blind sac. This migrates dorsad and separates from the olfactory lobe so that the Nervus olfactorius extends anteriorly to the sac. At the

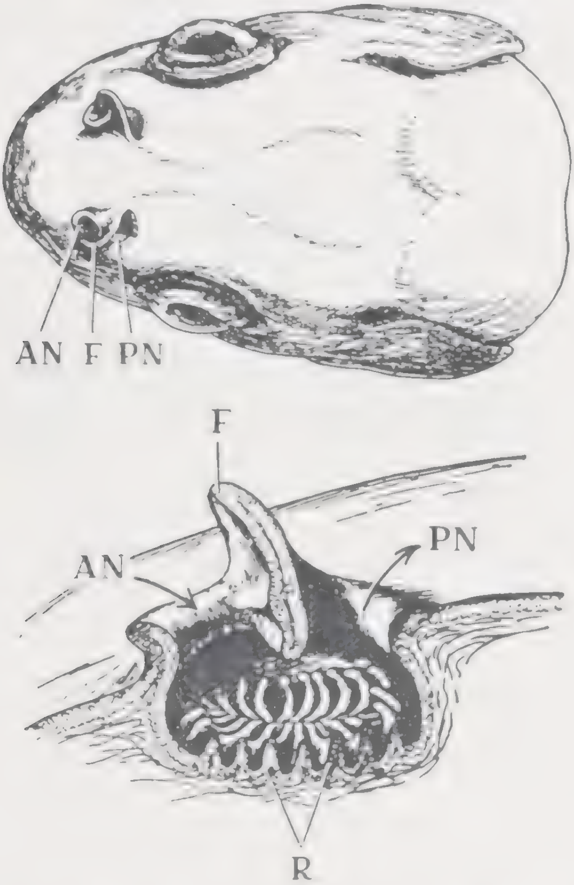

FIG. 1. Position of nares and dissection of olfactory capsule of the minnow, *Phoxinus* (from von Frisch, 1941). AN: anterior naris, PN: posterior naris, F: flap, R: olfactory rosette.

21-mm. stage, the olfactory organ divides into two, fuses, and develops folds and later the sac opens to the outside.

In the teleost fishes, the paired olfactory pits are almost always on the dorso-anterior aspect of the head and are in no direct way connected with the respiratory current (Fig. 1). In nearly all fishes each pit has two entirely separate openings, an anterior inlet and a posterior outlet. By means of these two openings, a current of water enters and leaves each pit.

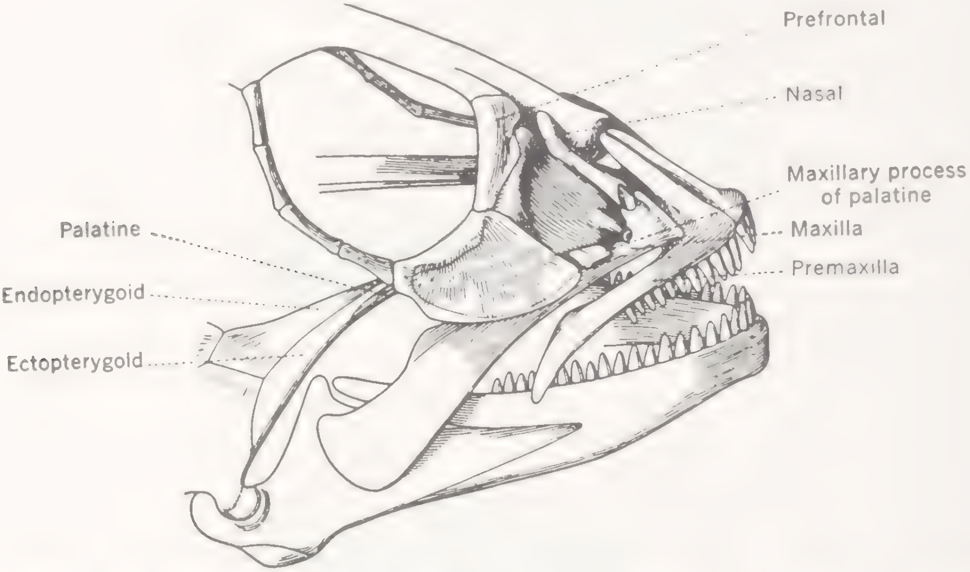

Fig. 2. Sketch of the skull of the blenny, *Zoarces viviparus*, showing the pouch-like nose which operates like a syringe (from Liermann, 1933).

Burne (1909) described the olfactory organs of 51 genera of fishes comprising 32 families. Pipping (1927) has classified olfactory mechanisms into three general types:

(1) Where water is deflected through a capsule as the fish moves through the water (*Esox lucius*, *Nerophis ophidion*, *Siphostoma typhle*, and *Sphaeroides maculatus*).

(2) Where breathing movements compress an olfactory pouch to draw water in and out of a single opening (*Cottus scorpius*, *Zoarces viviparus*, *Pygosteus pungitius*, *Gasterosteus aculeatus*, and *Scophthalmus maximus*; see Fig. 2).

(3) Where water circulates in through the anterior aperture and out the posterior, whether caused by breathing movements, cilia or both (*Rutilus rutilus*, *Scardinius erythrophthalmus*, *Phoxinus phoxinus*, *Perca fluviatilis*, *Acerina cernua*, *Gobius niger*; see Figs. 3, 4, and 5).

An unusual anatomical form was described in *Zeus faber*, the John Dory, by van den Berghe (1929). In this fish the olfactory rosette is suspended by a fold in the nasal cavity. Respiratory movements compress surrounding lymphatic sinuses which in turn act upon the nasal cavity in such a way as to force the rosette against the anterior naris, blocking and opening it alternately like a valve. Ducts connect these sinuses with other lymphatic sinuses of the head.

Pipping (1927) observed flow of water in and out of the nasal pit of a stationary roach (*Rutilus rutilus*). Cilia draw water through the anterior opening and propel it out of the posterior opening. In the perch (*Perca fluviatilis*) the nasal pouch is operated when surrounding bones (Fig. 3) compress it during respiratory movements. Liermann (1933) and Pipping noted that carmine particles were taken in via the anterior opening and expelled through the posterior. It appears to be a very weak suction, but unidirectional none the less. Both Pipping (1927) and Wunder (1927) established odor perception in the European perch.

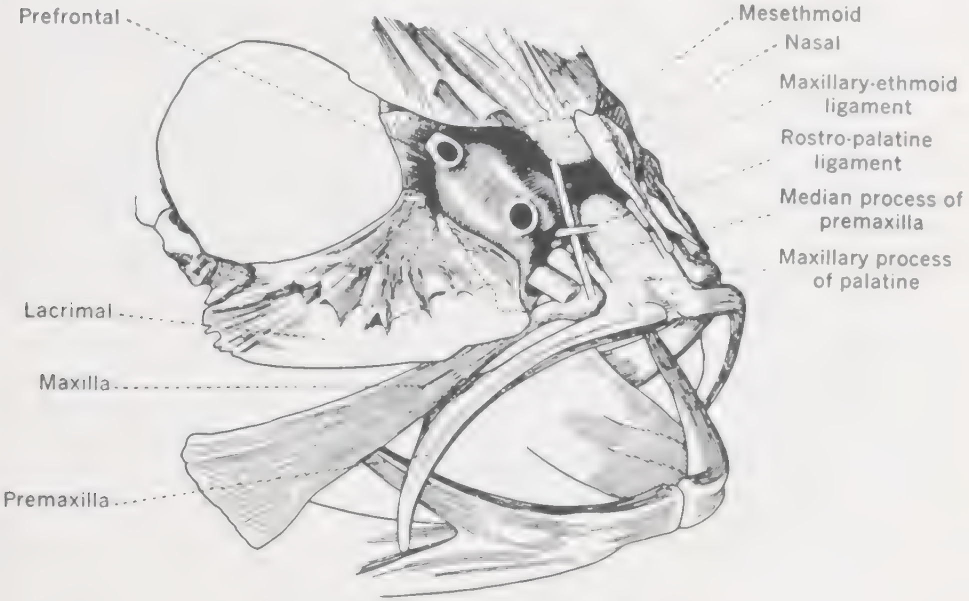
Prefrontal
Lacrimal
Maxilla
Premaxilla
Mesethmoid
Nasal
Maxillary-ethmoid ligament
Rostro-palatine ligament
Median process of premaxilla
Maxillary process of palatine

FIG. 3. Skull of the perch, *Perca fluviatilis*, with the olfactory organ emphasized (from Liermann, 1933).

The eel (*Anguilla anguilla*) has a very elaborate development of the nasal apparatus (Fig. 5). It is elongated, with many folds extending laterally. Ciliary action alone is responsible for flow of water over these many folds of the epithelium according to Pipping and Liermann. A specialized structure with enlarged surface area, such as this, obviously allows many more sensory cells to be activated.

Gross observations of the anatomy of *Oncorhynchus kisutch*, the coho or silver salmon, by the author would classify it in Pipping's group I, although histological sections may later show the epithelium to be ciliated. Certainly the main flow of water over the olfactory tissues is produced by swimming movements or by currents in the stream. But here also, no observations have been made to determine how breathing movements influence the flow of water.

The gross and microscopic anatomy of the nose has been related to the ecological habits of the fishes by Teichmann (1954). He classified them into three groups: (1) Those species in which the eye and the nose are well developed such as in schooling and nonpredatory fishes e.g. *Phoxinus*, *Gobio*. (2) Species in which the eye is better developed than the nose such as microsomatic daylight predators e.g. *Esox*, *Gasterosteus*. (3) Those in which the nose is exceptionally well developed compared with the eye as in macrosomatic solitary predators, e.g. *Anguilla*, *Lota*. He illustrated also the range of development of the nasal epithelium by a diagram in which the folds of the epithelium were sketched for 10 different species and ranged in number from 2 in the stickleback to 68–93 in the eel. The minnow (*Phoxinus*) had a median

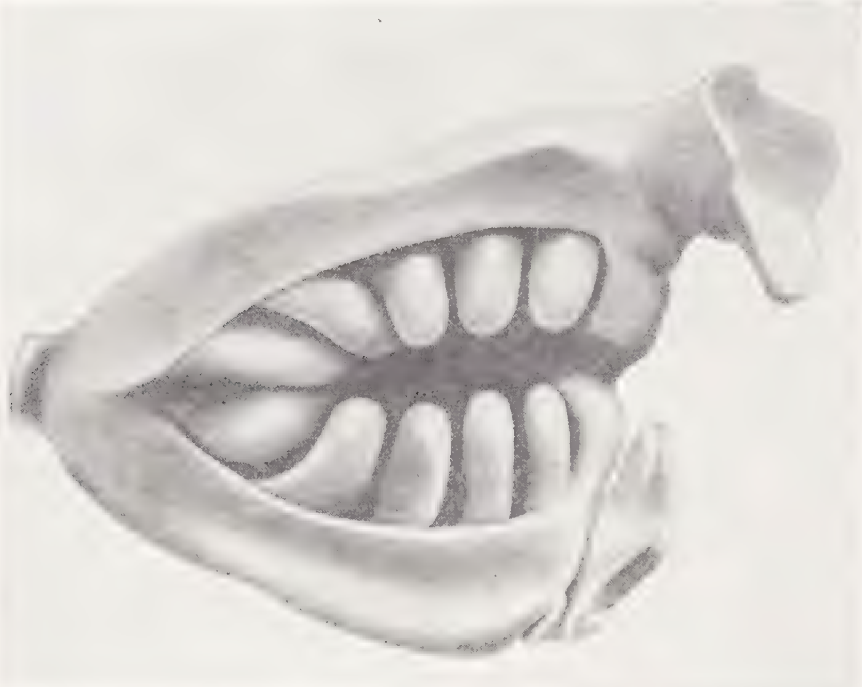

FIG. 4. A model of the olfactory rosette of the perch (× 32) (from Liermann, 1933).

position with 11–19 folds and was listed as belonging to (1) above.

The histological structure has not been described in many species, although Adrian and Ludwig (1938) have pictured the ciliary nature of the epithelium and Blaue (1884) showed olfactory buds lying alongside stratified epithelial cells. Jagadowski (1901) described sensory hairs in *Esox lucius* whose distal ends reach through the olfactory mucus, but this observation needs verification with modern techniques.

Allison (1953) reviewed the structure of the peripheral and central divisions of the olfactory and vomeronasal formations in vertebrates in the light of recent experimental studies, stressing possible functional significance. To a modern reviewer, the paucity of precise anatomical and histological descriptions for common North American species of fishes is a surprising void in the literature. Many student projects in universities could be profitably devoted to such descriptions.

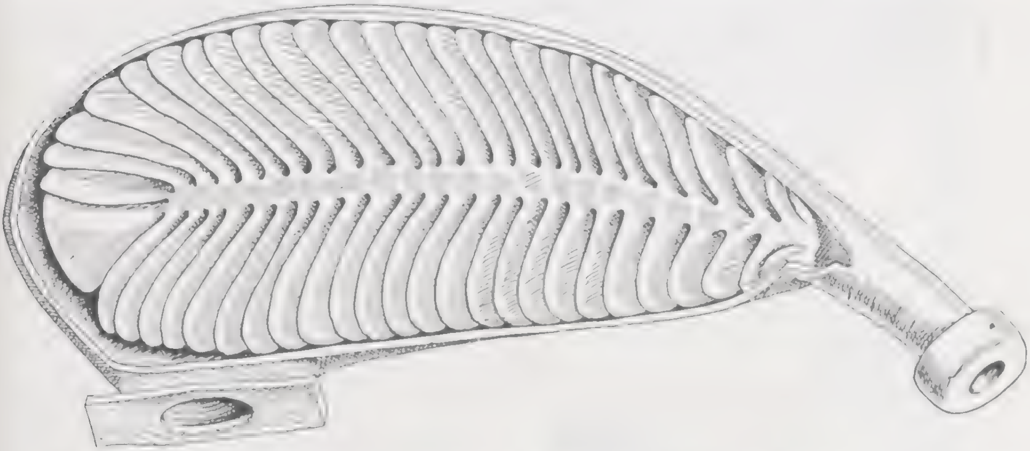

FIG. 5. Model of olfactory rosette of the eel (× 20) (from Liermann, 1933).

## IV. IMPULSES FROM OLFACTORY TISSUE TO BRAIN

In certain fishes (bullhead *Ameiurus*, carp *Cyprinus*, and tench, *Tinca*) the olfactory bulb is connected with the forebrain by a nerve strand (the olfactory stalk) (Fig. 6), which measures about 2 cm. long and is composed of from 500 to 1000 medullated fibers. Adrian and Ludwig (1938) studied the action potentials in the olfactory stalk of the bullhead during stimulation of the olfactory end-organs. Potential changes of small amplitude passed up the stalk as long as the preparation survived, though nothing but distilled water had been introduced into the sac. This resting discharge was of very low frequency. When the sac was irrigated with fluid containing small fragments of some odorous material, e.g., putrefying earthworms, a burst of impulses occurred at high frequency after a latent period of from 0.5 to 5 seconds or longer. The irrigation was more powerfully stimulating if the fluid contained small fragments of the material than if it had been filtered. Mechanical stimulation, caused by pressing upon the roof of the sac, or by touching its interior with a brush, was also found effective. The nervous discharge

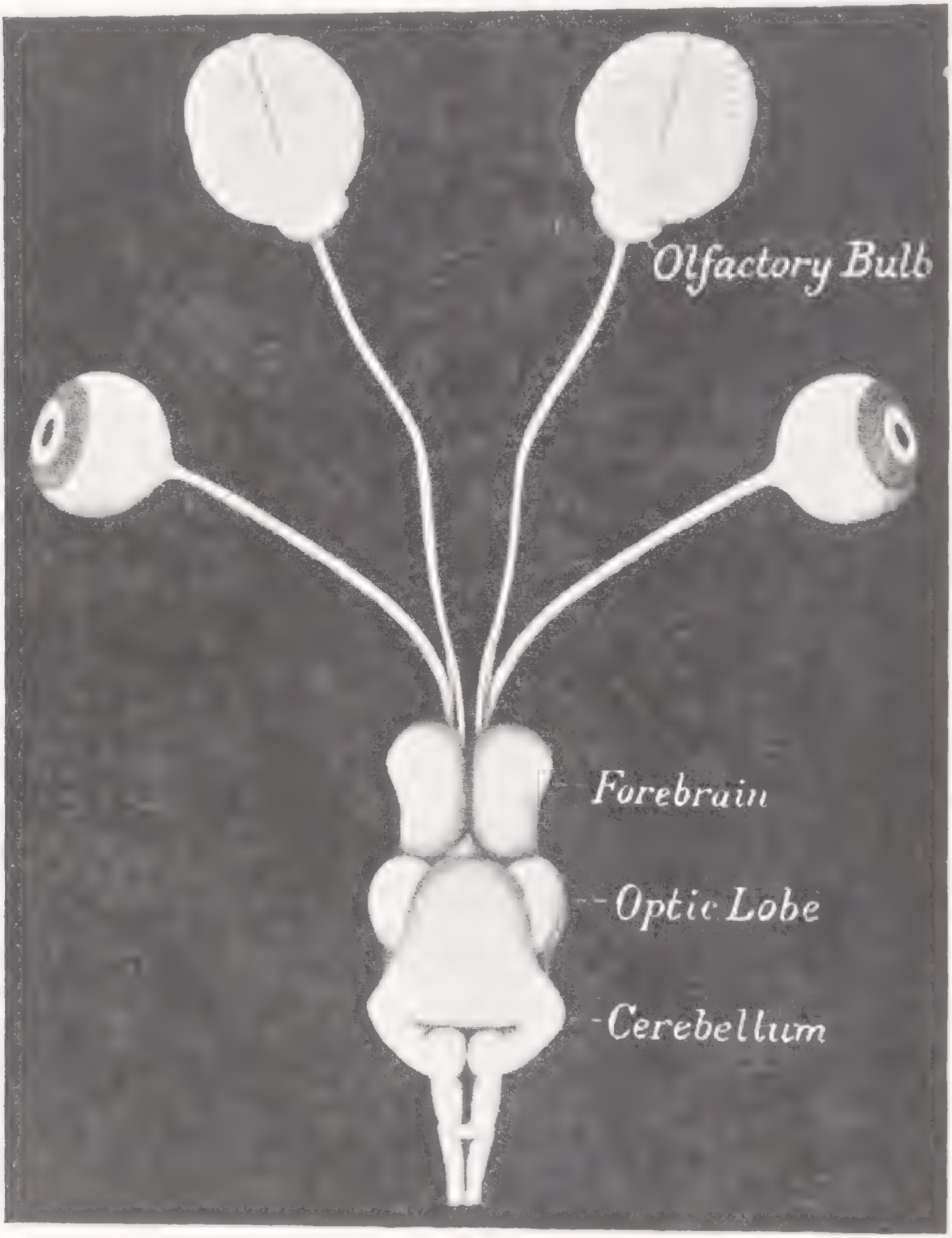

FIG. 6. The olfactory bulb of the bullhead (*Ameiurus*) and its relation to the forebrain (from Adrian and Ludwig, 1938).

was not rhythmical, there being no evidence that groups of fibers discharged synchronously.

In *Carassius*, experimental observations by Sanders (1940) indicate that some olfactory fibers must pass to the optic tectum because second-order responses are abolished by extensive lesions in the tectum.

## V. TASTE AND COMMON CHEMICAL SENSE

The distribution of taste buds in terrestrial vertebrates indicates the presence of this sense in the mouth region even in the amphibians. The anatomy of the gustatory elements in fishes is briefly discussed by Evans (1940). In the cod (*Gadus*) Haller von Hallerstein (1934) depicts, in a beautiful diagram, the innervation of the mouth, pharyngeal, and gill area from branches of the IX and X cranial nerves. It may be presumed that they conduct impulses from the taste buds. Reviewing the literature for fishes, Herrick (1903) lists over 35 species in which taste buds are known to occur on the outer surface of the animal as well as in the mouth. Among them are the carp *Cyprinus*, sturgeon *Acipenser*, cod *Gadus*, ling *Lota* and mullet *Mugil*. The bullhead *Ameiurus* is remarkable in this respect in that its whole outer surface is provided with these organs, which are most abundant on the barbels. These taste buds are innervated by branches of the N. facialis (VII), N. glossopharyngeus (IX), and N. vagus (X); these have their center in the medulla oblon-

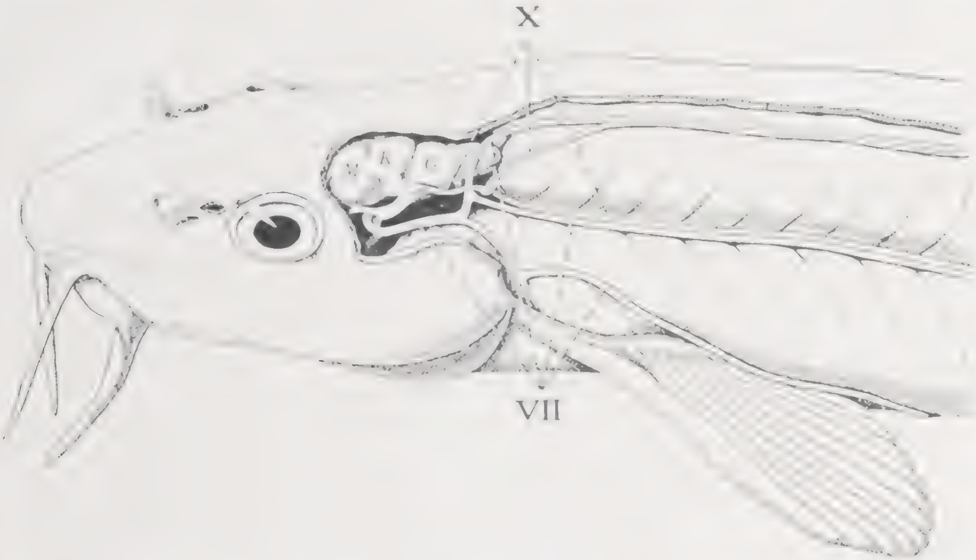

FIG. 7. Dissection of X and VII cranial nerves of *Nemacheilus*, which supply the nerve endings of the taste and common chemical sense system (from Dijkgraaf, 1934).

gata (Herrick, 1906). Figure 7 (from Dijkgraaf, 1934) shows that the innervation of the flank taste receptors of *Nemacheilus* is from branches of cranial nerves VII and X.

When a piece of meat is brought into contact with the barbel of a bullhead, the animal will immediately seize and swallow the morsel. The same is true when the meat is brought in contact with the side of the fish. This quick seizure and swallowing of the food has been called by Herrick the gustatory response. The same form of response is made to meat juice discharged from a pipette on the side of the fish. That these responses are really gustatory is shown by the fact that when the branch of the seventh nerve that innervates the taste buds on the flank of *Ameiurus* is cut, the responses no longer occur (Parker, 1912).

Conditions similar to those in *Ameiurus* were recorded by Herrick in a number of other fishes, and it is thus clear that taste is a general integumentary function in many of these animals.

Nagel's (1894) observations on *Amphioxus* were confirmed by Parker in 1908 when it was shown that the skin of this lower chordate was sensitive to solutions of acids, alkalis, alcohol, ether, chloroform, turpentine, oil of bergamot, and oil of rosemary, but not to solutions of sugar. Parker also demonstrated that the skin of *Ameiurus* was sensitive to sour, saline, and alkaline solutions, a condition that he subsequently found to be true for the ammocoete larva of the lamprey (Parker, 1908, 1912).

*Nemacheilus barbatulus* lost its ability to perceive tastes through its flank receptor when Dijkgraaf (1934) severed the appropriate branch of the N. facialis (Fig. 7). In 1935, Scharrer described the perception of acids and of sardine extract by sensory cells in the free fin-rays of *Trigla;* with others (1947), he reported on taste responses in two other marine fishes.

These observations warrant the general conclusion that the outer surfaces of most fishes are receptive to stimulation by chemical substance of a mildly irritating kind.

Early works on food acceptance as related to taste by Aronsohn (1884), Steiner (1888), and Baglioni (1909) are of little consequence to this review. Sheldon (1909, 1911) demonstrated in the dogfish that the sensitiveness of the nostrils of this fish to weak solutions of oil of cloves, pennyroyal, thyme, and the like, was not influenced by severing the olfactory crura, but disappeared on cutting the combined maxillary and mandibular branches of the trigeminal nerve. Evidently the nasal surface of *Mustelus*, like those of the higher vertebrates, are innervated by fibers from the trigeminal nerve.

Wunder (1927, 1936) studied the perception of natural foods by taste buds located on the body, barbels, lips, and gill-rakers. He described in particular a large, well-polstered pad on the roof of the carp's mouth, whose entire surface is strewn with taste buds enabling it to take in miscellaneous items, from which it appears able to sort the food organisms from detritus. The action of acids on taste receptors in the mouth of *Phoxinus* was studied by Klenk (1930).

Strieck (1924), in von Frisch's laboratory, removed the forebrain of the minnow, *Phoxinus phoxinus*, thus destroying the ability of the fish to be trained to odors. He then initiated a training regime so that the fishes should learn to anticipate food when presented with cotton wads soaked in sugar solution, but no food if given salty, bitter, or sour substances. Such an olfactory-excised fish, after training, would energetically search the bottom for food each time the training taste was presented on a cotton wad, and ignored any other taste. He established thresholds of 10 to 11% for grape sugar, 6% for salt (NaCl), 0.15% for acetic acid, and 0.02% for quinine. Later, Trudel (1929) attempted to discover if other tastes that are sweetish for man are also detected by fish. For negative training, he used punishment such as presenting broken glass in the cotton wad, applying a blow with a glass rod, or an electric shock, or even rapping on the aquarium. Trudel taught his minnows to discriminate between several natural sugars. They even responded to saccharin. Quinine was perceived in dilutions as low as 0.0025%, whereas his human subject had a threshold of 0.0003%.

Following this work, Krinner's (1935) trained minnows detected NaCl at dilutions of $4 \times 10^{-5}$ *M* and saccharose at $2 \times 10^{-5}$ *M*. He found their sugar thresholds to be 512 times lower than those for man and their salt thresholds 184 times lower. Removal of olfactory tissue did not change these thresholds. Therefore, a true gustatory sense was verified. Hunger motivation and long training were essential features of Krinner's experimental discipline.

This differential type of test using conditioned responses gives more decisive and critical proof of the ability of the animal to discriminate between odorous materials than did the spontaneous responses recorded by earlier workers.

Copper acetate was recommended as a shark deterrent by Burden (1945); this substance is irritable, no doubt, to the common chemical sense. This work needs reverification under a more rigid experimental design. Our preliminary laboratory tests showed it to be toxic, but not repellent to salmon. Whiteley and Payne (1947) have also tested shark repellents.

## VI. OLFACTION IN FISHES

### A. Perception of Food Odors

Parker (1910) allowed five normal bullheads (*Ameiurus*) to swim in an aquarium in which were hung two wads of cheesecloth, one of which contained concealed earthworms. In the course of an hour the wad containing the worms was seized 11 times by the fishes, even though it was periodically interchanged in position with the other. During the same period the wad without worms was passed over by the fishes many times and never excited any noticeable reaction (Parker, 1922).

Ten bullheads were next prepared for further experimentation; in five of these the olfactory tracts were severed, and from the remaining five the barbels, the seat of the chief external gustatory organs, were removed. After the fishes had recovered from these operations, they were put in an aquarium into which was introduced a wad of cheesecloth containing minced earthworms. During the first hour, the wad was seized 34 times by fishes without barbels but with normal olfactory organs; whereas, although fishes with cut olfactory tracts often passed over the wad, it was never seized by any, and was "nosed" only once by one of them.

Recently in our laboratory, a student project, using a technique discussed later in this review, established the ability of the minnow *Hyborhynchus* to discriminate between three species of aquatic invertebrates, *Gammarus fasciatus*, *Hesperophylax* sp. (a caddis), and *Hyalella*.

Sheldon (1911) closed the nares of the dogfish *Mustelus* with cotton plugs and, in 1914, Parker showed that when only one nostril is thus plugged, the fishes turned persistently toward the side of the open nostril. Such responses indicate that, in the seeking of food under normal conditions, dogfishes, and probably other fishes as well, turn toward the side on which the concentration of odorous particles is greater. In criticism of this conclusion, it has been pointed out that the animal may as likely have turned *away* from the irritating plug as *toward* the open nostril. In the hammerhead shark *Sphyrna* the nostrils, as well as the eyes, are carried on remarkable lateral projections that extend sideways from its head; this may facilitate the localization of odors.

Olmsted (1918) used sixty *Ameiurus* at different seasons and charted the number of bites by two fish during half-hour trials with various natural substances. Grading the most active material on the basis of 100, he found earthworms high, liver next, and human saliva third. He observed, "But since human saliva proved fairly stimulating to the fish (bullhead), the time-honored custom of 'spitting on one's bait' does seem to be more than superstition, and perhaps for this reason may receive the sanction of science."

### B. Recognition of "Body" Scents

Wrede (1932) built a simple three-compartment maze. A fish was placed in one of the two small sections for a few minutes, then removed. A test minnow (*Phoxinus*) introduced into an adjoining compartment could sniff at two apertures and enter either. It was only fed if it entered

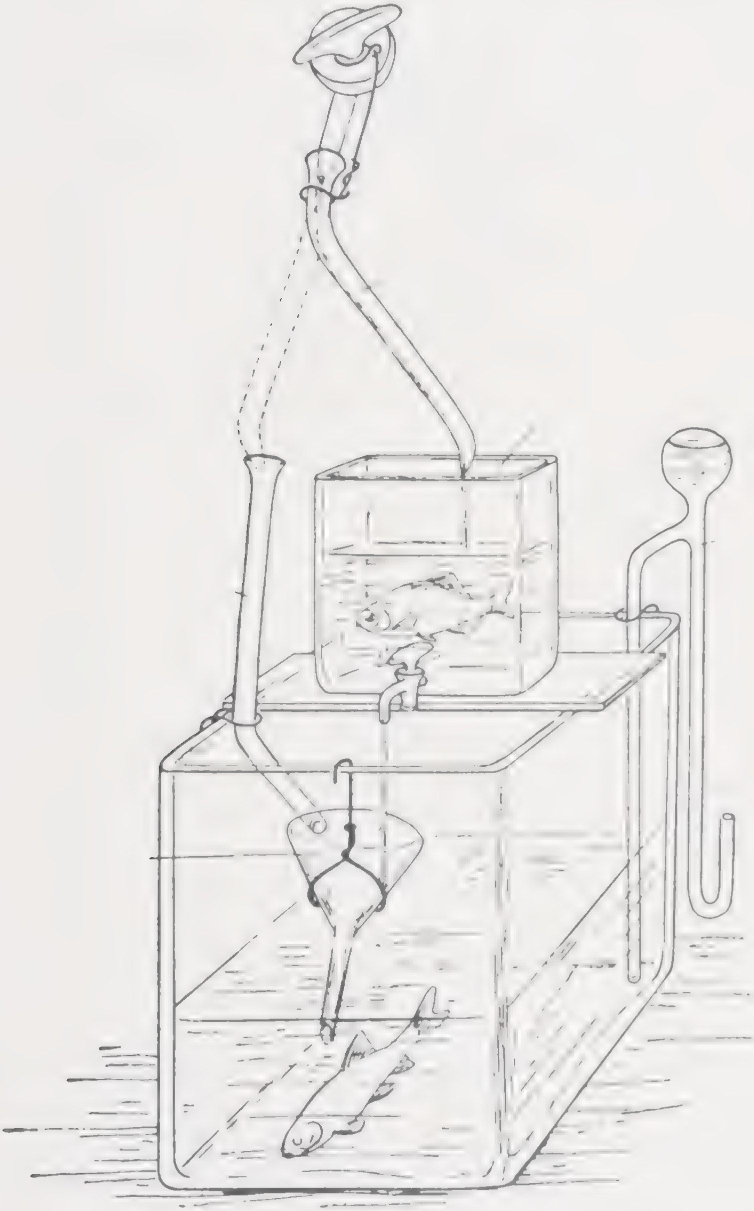

FIG. 8. Arrangement used to train fishes to give a feeding reaction when exposed to the odor of another species (from Göz, 1941).

the positive section and soon learned to associate the odor of a given species with the correct compartment.

Following up this work by Wrede, Göz (1941) designed a testing apparatus (Fig. 8), which made possible conditioned response experiments which showed the minnow's ability to discriminate, by odor, 15 different species of fish representing 8 families. (Positive: reward by feeding; negative: punishment by a light blow from a glass rod). Precautions were taken against collateral cues. By positive and negative training, his fish learned to differentiate between closely related species within its own family and could discriminate a training odor even if it was mixed with those of other species. Göz claimed to have shown further that individuals within a school have an individual body odor recognizable by each member of the school.

A fright reaction was elicited in the minnow by odors from *Perca fluviatilis*, *Salmo trutta*, and *Ameiurus nebulosus*. Blinded minnows which did not react to pike (*Esox*) odor, were placed in with a pike who captured some of them. The remainder were again tested and a combination pike and minnow odor then induced a fright reaction. Thus the negative reaction of the minnows to *Perca fluviatilis*, *Salmo trutta*, and *Ameiurus nebulosus* may have been the result of traumatic experiences with these three species and not, therefore, an example of innate response.

### C. Alarm Substances

Strong fright reactions were observed by Noble (1939) in young *Tilapia* to some substance released from injured fish skin; he gave no quantitative data but merely mentioned the fact in passing in a general article on animal behavior.

In a methodical series of experiments, von Frisch (1941) examined the nature of a substance he calls an alarm substance (Schreckstoff). When an injured minnow (*Phoxinus phoxinus*) was placed among its school, they would exhibit an alarm reaction ("Schreckreaktion"). The school would concentrate after a latent period of 0.5 minutes and immediately seek cover or flee. The alarm reaction is incited by a repellent substance given off by the injured skin. When a solution of 0.1 g. of fresh skin in 100 ml. of water was poured into aquaria of 25- to 150-liter capacity, positive reactions were recorded. There was some variability between different schools of fish; the most satisfactory reactions were recorded when schools were kept in aquaria to which they had been conditioned for some time. No alarm substance was found in extracts of intestine or liver. The ovaries were only one-hundredth, muscle one-twentieth, and gills one-fifth to one-tenth as active as skin. When the olfactory lobe is destroyed or the olfactory nerve severed the minnows do not respond to the substance; it must, therefore, be odoriferous. Control fish showed that the operation, *per se*, was not a complicating factor. The skin of dead minnows retains its activity for several days.

The activity of extracts from forty-one species of freshwater fishes was tested and compared with that from the minnow. Eighteen species belonged to families other than the Cyprinidae; their skins contained no appreciable quantities of the alarm substance for the minnow. The skin extracts from other Cyprinidae were active in general. The reaction was observed on two occasions in nature; once when a school was attacked by perch and again when it was disturbed by a bittern. The minnows took food at a point some distance from the area of alarm but behaved more nervously than when they are thoroughly conditioned.

In a recent detailed study of the distribution of the "Schreckstoff," Schutz (1956) found that sixteen species of European Cyprinidae and five south Asiatic species responded to this substance from the skin of their own species. All the European species responded to that of all other species used. Only the females of *Heterandria* and *Lebistes* (Poecilidae) responded to the skins of both male and female of their species. Schutz found that the reaction developed late in the life of the young and appeared only after schooling behavior had been established. The substance is nevertheless present in the skin and may induce the reaction in older individuals. Schutz obtained the fright reaction when a suspension of 0.01 $mm^2$ of skin (0.002 mg. net weight) in 100 ml. of water was presented to a school of fish in a 14-liter aquarium. If one assumes the concentration of the substance to be 0.1% of the wet weight, this represents a threshold of $2 \times 10^{-11}$ ignoring the dilution in the tank. He found fatigue occurs very quickly, often previous handling is sufficient to destroy the response but there is great individual variation.

These experiments and observations help to explain the development and maintenance of schooling behavior among nonpredatory fishes, because of the general survival advantages that the group must receive from an alarm or repellent substance given off by an injured comrade.

### D. Repellents

Recently, Brett and MacKinnon (1952) poured rinses of human hands into a salmon ladder, during an autumn migration of adult fish. These rinses proved surprisingly repellent to the ascending coho salmon. Control fluids such as water, urine, and tomato juice were ineffective.

Hiatt *et al.* (1953) have recently tested large numbers of artificial substances for their repellent action on fish and established thresholds for them. Human skin irritants, lachrymators, and nerve poisons are irritable to fishes. Multihalogenated organic compounds, organic thiocyanate, and halogenated ketones disperse fish in schools, some are active in concentrations as low as 0.05 p.p.m. Their effect is not necessarily olfactory.

### E. Discrimination of Odors of Aquatic Plants

Walker and Hasler (1949) used the apparatus in Fig. 9 for quantitative recording of scores made by fish on a conditioned response training regime; this new testing procedure gives reproducible results. We undertook to investigate the role of odors of aquatic plants in the life of fishes. We showed that blinded bluntnose minnows (*Hyborhynchus notatus*), in groups of six and eight, were able to discriminate, after a period of

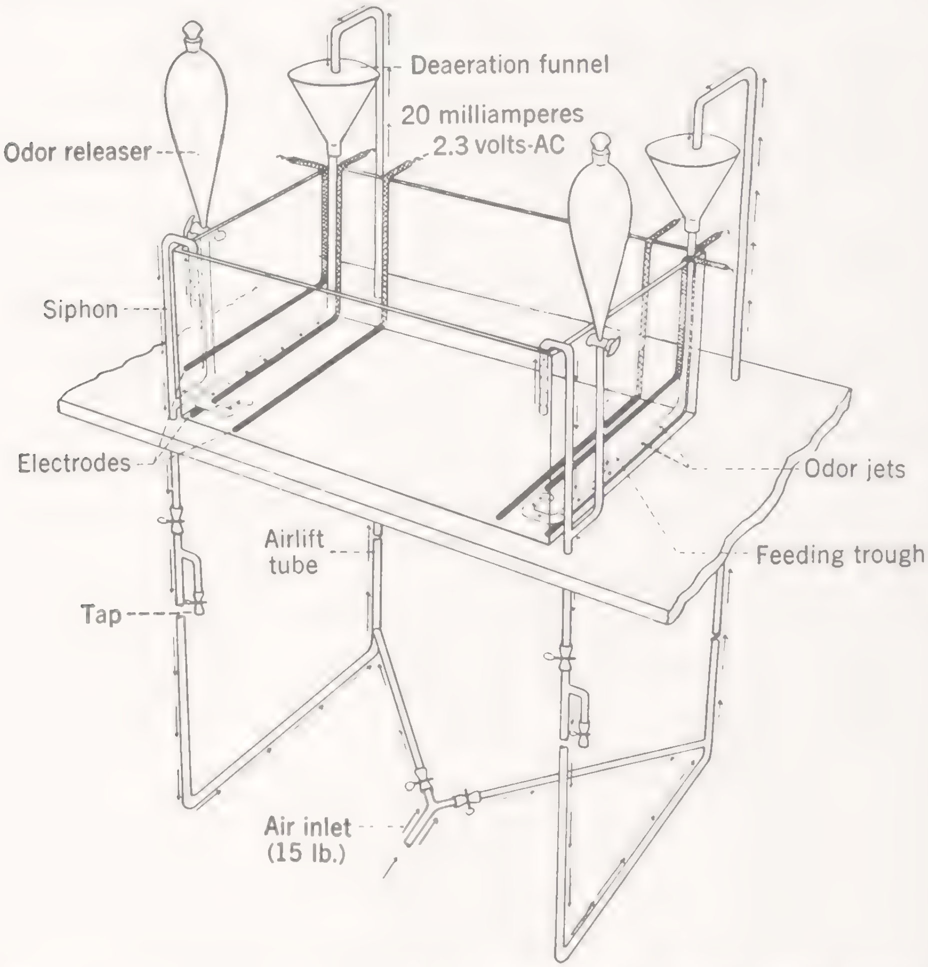

FIG. 9. Apparatus for conditioning fish to discriminate odors (from Hasler and Wisby, 1950).

training of two and one-half months, between the odors of *Myriophyllum exalbescens* and *Ceratophyllum demersum*, when food was the reward and electric shock was the punishment. Reverse training substantiated this.

Similarly, these fish were trained to discriminate odors of other sets of plants: (a) *Ranunculus tricophyllus* and *Anacharis canadensis*; (b) *Utricularia vulgaris* and *Vallisneria americana*, (c) *Potamogeton zosteriformis* and *P. crispus*; (d) *P. amplifolius* and *P. vaginatus*. Rinses containing odors of other plants were introduced into aquaria of fishes so trained, in a Latin-square design by a generalization test. Of 12 plant species tested, the odors of only 2 seemed to resemble each other; that is, when *C. demersum* had negative significance, the fish associated with it the odor of *A. canadensis*. Trained minnows whose olfactory epithelium had been destroyed by heat cautery were unable to respond to plant rinses, thereby proving that the rinses contained a stimulating scent detected by sense of smell.

When minnows were fatigued by or adapted to a strong rinse of one species, they still detected odors of other species when these were superimposed. The minnows were able to detect a water rinse of a sprig of aquatic plant diluted 1:10,000 after it had been further diluted by streaming it into a 7-gallon aquarium, thus demonstrating a very high degree of odor-sensitivity.

The results of these studies lend support to the view that aquatic plants may well play an important role in the life of a fish. Their odors may guide fish into feeding grounds, since many fishes commonly feed in turbid water, at dusk, at dawn, and at night, when visibility is poor. Moreover, the odors may serve as attractants to immature fishes in littoral areas of lakes and prevent them from straying from cover.

### F. Discrimination of Pure Chemicals

Hasler and Wisby (1950) explored the application of their technique to biological assay of pollutants. Phenol, a common pollutant in municipal water supplies, cannot be readily detected in low concentrations until it has reacted with chlorine forming odorous chlorophenols. A method was presented in which bluntnose minnows (*Hyborhynchus notatus*) were used to detect the presence of phenols in concentrations below the threshold for man (0.01 p.p.m.). Fishes of two aquaria were trained to associate the odor of phenol with food and the odor of a *p*-chlorophenol with punishment. The minnows of two other aquaria were trained to the same odors, but with reverse meanings. Upon completion of the training period, the minnows were discriminating successfully between these two substances at concentrations of at the most

$5 \times 10^{-4}$ p.p.m. A generalization test described by Hilgard and Marquis (1944), which was used to test *o*-chlorophenol, the third odor, indicated that the fishes were able to distinguish it from the two training odors. The biological assay of phenolic compounds by this method is primarily one of detection and not of quantitative analysis.

*Phoxinus* were trained by Neurath (1949) to discriminate eugenol and phenylethyl alcohol. Trained fish detected eugenol at dilutions of $6 \times 10^{-7}$ p.p.m.; phenylethyl alcohol at $43 \times 10^{-9}$ p.p.m. He found that trained specimens were unable to discriminate these compounds after the olfactory lobes were extirpated, although they would still respond to taste substances such as quinine and acetic acid.

Recently, Tester (1952) and van Weel (1952) have been examining the question of simulated bait attractants for tuna. Water extracts of tuna induced feeding reactions whereas extracts of white-fleshed species did not. It is still not clear if the fish had been inadvertently conditioned to tuna. The maintenance and testing of such large animals is fraught with difficulties.

## VII. ORIENTATION TO THE PARENT STREAM AS IT RELATES TO ODOR RECOGNITION

That salmon usually return to spawn in the stream or tributary in which they were reared, is acknowledged as fact by most modern fisheries biologists. Although previous workers who have investigated this phenomenon have suggested that chemical stimuli may influence the movements of salmon, no one has been able to guide salmon to an artificial stream.

Hasler and Wisby (1951) postulated that river and creek waters contain some characteristic odor to which young salmon become conditioned while in the stream, and which they recognize and orient to upon reaching the parent stream as mature migrants. This theory embodies the principle that a salmon returning to its parent stream reacts differently to the odor of that stream than to any other. In order for a salmon to return to its home stream there must be the possibility of a differential reaction, not a simple response to a repellent or an attractant. This guiding odor must remain constant from year to year and have meaning only for those salmon which were conditioned to it during their early freshwater life.

This theory presents three distinct problems:

(1) Do streams have characteristic odors to which fish can react? If so, what is the nature of the odor?

(2) Can salmon detect and discriminate between such odors if they do exist?

(3) Can salmon retain odor impressions from youth to maturity?

In order to answer the first question, a group of bluntnose minnows (fish of proven olfactory acuity), were trained to discriminate between the waters of two Wisconsin creeks. That scent-perceiving organs were the sole means of discrimination in these tests was proven by destroying the olfactory tissue of trained fishes, after which they no longer responded to the training odors.

Chemical analysis of the stream waters indicated that the only major difference between them was in the total organic nitrogen fraction. Experimental evidence to substantiate this was obtained by separating the water into various fractions and then presenting these to trained fishes. The fish trained previously to natural water did not react to the inorganic ash, or to the distillate or residue of water fractioned at 100° C. However, they recognized the distillate, but not the residue, of water fractionated by vacuum distillation at 25° C., a strong indication that the odorous stimulant is a volatile, organic substance.

In a test of the retentive capacities of the trained minnow, it was determined that even this fish, which is not specialized in this respect, could differentiate between the odors for a comparatively long period after cessation of training. Learned behavior was found to be retained longer by young fish than by old.

The method of training, which was used with such success with the minnows, was then applied to salmon fry, *Oncorhynchus kisutch*. After a short period of training it was evident that these fish too could discriminate between the odors of the two Wisconsin creeks.

It was proposed by Hasler and Wisby (1951) to employ an artificial substance to which salmon fry could be conditioned and which could then be used to decoy them, upon their return, into rehabilitated streams, formerly dammed or polluted, or to salvage a run which would not be able to pass a newly constructed power dam. Such an odor must be neither a repellant nor an attractant for unconditioned salmon.

Wisby and Hasler (1952) designed an apparatus to test the reactions of unconditioned salmon to various organic odors which might be used for the above purpose. This unit consists of four arms which converge on an enlarged central compartment. Water is introduced into the upper end of each arm and cascades down a series of eight small falls until it enters the center compartment, from which it exits through a drain. The entrance to each of the four arms is guarded by a gate, all of which can be raised by pulling a single cord.

Salmon fry were placed in the center compartment and, upon introduction of an odor into one of the arms, the gates were raised permitting the fish to enter the arms. Their distribution after the test was then noted and compared with the distribution obtained with no odor present.

Many organic odors were presented to unconditioned salmon and their reactions noted. None was found which would attract salmon, many seemed not to be perceived, and the remainder were distinctly repellent in action at the concentrations used. Of the latter, only two were deemed suitable for further testing. One of these, dicyclopentadiene, proved to be so insoluble as to render accurate dilutions impossible.

It was observed that *Oncorhynchus* fry were not totally repelled by dilute solutions of morpholine, although they could easily detect it in concentrations as low as $1 \times 10^{-11}$ p.p.m. Although the minimum detectable concentrations were not established, the concentrations presented indicate a sensitivity to chemical stimulation far exceeding that reported for any fish tested to date.

It appears, then, that the compound morpholine fits the requirements mentioned previously. It is soluble in water, thus permitting accurate dilutions; it is detected in extremely low concentrations, thus making the treatment of large volumes of water feasible; and, it is chemically stable under stream conditions. Furthermore, at these low concentrations, it is neither an attractant nor a repellent for unconditioned salmon and thus should have meaning only for those salmon previously conditioned to it. Field tests should be conducted to determine whether salmon fry and fingerlings which have been conditioned to morpholine can be decoyed to a stream other than that of their birth, upon their return to fresh water as mature migrants.

Craigie (1926) considered the nasal cup to be an aid to orientation in salmon. He captured *Oncorhynchus nerka* at sea and cut the olfactory nerves by a radial incision across the snout. The homing stream was believed to be the Fraser River but was not certainly known and there were insufficient recaptures to show clearly a disrupted migratory path.

Wisby and Hasler (1954) captured sexually ripe coho salmon, *Oncorhynchus kisutch*, at two branches of the Issaquah River in Washington and returned them downstream below the fork to make the run and selection of stream again. In half of them the nasal sac was plugged with cotton. The great majority of normal fish selected again the stream of first choice, while the plugged-nose fish returned in nearly random fashion. No comparable pressure trauma was applied to the control fish; nevertheless, this experiment is indicative of the important role that the functional olfactory system may have in orientation.

## VIII. CONCLUDING COMMENT

Only intensive and imaginative studies in the future can show the subtle role of the sense of smell in fishes. The vast degree of morphological variation, the many different kinds of habitats, and the manifold community relationships present a wealth of experimental material and problems, the exploration of which can certainly advance our knowledge of olfaction.

With regard to the orientation of homing fishes we need to answer the questions: Can fishes discriminate water masses sufficiently to stay within their boundaries? Can they "learn" several other stream odors besides the one of their parent river? Do specific points within a stream have characteristics "preferred" by olfactory discrimination? How are the cues from odors related, in biological meaning, to cues from sight and hearing? Are these cues that induce specific reactions meaningful only when they come in combinations or in succession?

Recent work in electrophysiology by Professor E. D. Adrian, Cambridge University, shows how chemicals in stimulating the olfactory tissues produce responses in different parts of the olfactory lobes of rabbits. This is indeed pertinent to an understanding of olfaction and it opens up a new field of investigation which should be extended to fishes and correlated with their innate responses and learning.

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## CHAPTER III: PART 1

# BEHAVIOR: CONDITIONED RESPONSES

H. O. BULL

Page
I. The Nature of the Conditioned Response . . . . . . . . . 211
  A. The General Theory of the Conditioned Reflex . . . . . . . . 211
  B. The Bearing of the Conditioned Response on the Interpretation of Higher Nervous Activity, and the Importance of the Ichthyopsid Type in This Respect . . . . . . . . . . . . . . . 214
  C. General Principles of Conditioned Response Formation in Fishes . . 215
II. Experimental Methods Used in the Formation of Conditioned Responses in Fishes . . . . . . . . . . . . . . . . . 216
  A. Apparatus . . . . . . . . . . . . . . . . . 216
  B. Procedure . . . . . . . . . . . . . . . . . 217
  C. Representation and Interpretation of Results . . . . . . . . . 218
III. Sensory Investigations by Conditioned Response Techniques . . . . . 218
  A. Sight . . . . . . . . . . . . . . . . . . 220
  B. Sound . . . . . . . . . . . . . . . . . . 220
  C. Smell and Taste . . . . . . . . . . . . . . . 221
  D. Current Flow . . . . . . . . . . . . . . . . 221
  E. Hydrogen Ion Concentration . . . . . . . . . . . . 222
  F. Salinity . . . . . . . . . . . . . . . . . . 222
  G. Temperature . . . . . . . . . . . . . . . . 222
  H. Pressure . . . . . . . . . . . . . . . . . 223
IV. The Relation of the Laboratory Study of Conditioned Responses to Behavior in Normal Environments, and Its Value to Fishery Investigations 224
  References . . . . . . . . . . . . . . . . . 224

## I. THE NATURE OF THE CONDITIONED RESPONSE

### A. The General Theory of the Conditioned Reflex

There are two distinct concepts of the "conditioned reflex": (1) That of Pavlov, Bekhterev, Watson, Liddell, and others, arising out of classical physiology, in which the "reflex" and derivatives from it has a neurological basis with specific physiological properties. In its extension, through the elaboration of conditioned reflexes, to higher nervous activity, this concept becomes highly speculative. (2) That of Skinner (1938), in which the neurological correlate of behavior is discarded. In this concept, the reflex is an analytical unit of behavior denoting only an observed relation between a stimulus and a response, of which the close coincidence is but one of its properties. What goes on between the two events, while a valuable and legitimate field of study in itself, does not (says Skinner) at present clarify, but tends to confuse "whole" behavior studies.

Both concepts, however, develop their general propositions along parallel lines. Organisms are regarded as being equipped with a wide variety of inborn specific reflexes—not necessarily present at birth—in which the stimulus–response relationships are governed by now well-established static and dynamic laws. There are many of these inborn reflexes known. They involve both smooth and striated muscle, vaso-motor systems, and glandular tissues. In humans, the patellar, pupillary, eyelid, and salivary reflexes are familiar examples. In fishes, motor responses involved in food getting, or reactions to an electric shock, have been generally used for experiments of this kind. These reactions may be evoked by previously neutral or unrelated stimuli, either internal or external, if the two are repeated in a constant time and circumstantial relationship. The procedure is called "conditioning" and the secondarily acquired action a "conditioned reflex" or "response." A distinction is to be drawn between the true conditioned response in which the conditioned stimulus usually precedes the unconditioned and the motivation is single, e.g. reward (food) *or* punishment (shock), and the differential responses obtained in discrimination experiments in which the two stimuli are given simultaneously and the motivation is both reward *and* punishment and its nature possibly more complex. This latter form has furnished a larger part of the evidence on sensory perception in some fields than has true conditioning.

The general idea of the conditioned reflex has been familiar in various guises for many centuries (see Cason, 1925). Four people share about equally in the distinction of having made the first modern objective laboratory experiment. It seems unlikely that Sherrington or Twitmyer knew of the work of the two Russians who appear to be the first authors, and yet they were almost certainly all working contemporarily on this kind of problem. Wulfson (1898), though calling the acquired reflex "psychic"—as also did Pavlov himself for a few years—wrote the earliest known paper. Liddell (1943), in the chapter on conditioned reflexes in Fulton's "Physiology of the Nervous System," brought to light an experiment of Sherrington's which may well have been made before Wulfson's. Sherrington had been studying the limits of cutaneous anesthesia in a dog using an inductorium with a noisy vibrator to give an electric shock. Six weeks after spinal transection the animal was being used for the study of vaso-motor reflexes, in the course of which closing the key of the same inductorium (though for test purposes only and not connected up with the animal) was followed by a conditioned cardiac inhibition, the heart beat falling from 180 to 54. This was found to be a stable conditioned reflex and clearly the result of the association of the noisy vibrator with the earlier unpleasant skin experiments. The actual term "conditioned reflex" was first used by Tolochinov (1902), reporting on work done in 1901. Twitmyer (1902) studied the knee jerk. In the course of it a bell had been sounded at the same time repeatedly as blows on the knee. During minor adjustments on the subject the bell was on one occasion sounded accidentally without the blow, but a jerk took place in all respects like the true reflex. He followed this up, confirming that the bell did in fact act in this way, and noting that the subject's kinesthetic sensations were also similar to those involved in the normal reflex.

In 1904 Pavlov was given the Nobel prize for medicine for his work on the physiology of digestion, and in this same year he published his paper on conditioned salivary reflexes in dogs, but calling them "psychic secretion" (Pavlov, 1904). He further reported on these studies in his Huxley Lecture in London in 1906, (Pavlov, 1906) but they remained almost unnoticed by the scientific world until he gave the Croonian Lecture to the Royal Society of London in 1928. From then onwards—in no small measure reinforced and to some extent publicized by Watson's "Behaviourism" (1925)—his theories became widely known and much discussed in many walks of life. Translations of his works also became available (1927, 1928, 1941). Nonetheless, Bechtereff (1912) and Kalischer (1907), both claimed to have "discovered" conditioned reflexes, and that their techniques were superior to Pavlov's. It came to be almost universally held that education, learning, acquired reactions, and indeed all forms of modifiable behavior were essentially the formation of conditioned reflexes of varying degree of complexity.

Lloyd Morgan, Herrick, Hilgard and Marquis, and many others prefer and use the alternative term "conditioned responses." Bechtereff and Mikhailoff (1920–23) call them "associated reflexes." For Pavlov a "natural" conditioned reflex was one built up with natural stimuli, e.g. food, as distinct from "artificial," e.g. shock; but for Mikhailoff a "natural" conditioned reflex was one formed in daily life as distinct from one built up during experiment, which was artificial. A "chain reflex" in the Pavlov sense is a "reflexo-complex" to Mikhailoff.

Much of the work of Thorndike, Lloyd Morgan and very many others in the study of animal association learning, and especially the larger part devoted to investigations of the reward–punishment type, is fundamentally similar to "classical" conditioning; it must all eventually converge into a common theory (see also Thorpe, 1950). Comprehensive treatments of the whole field are to be found in Hilgard and Marquis (1940), Konorski (1948), and Razran (1933, 1937).

## B. The Bearing of the Conditioned Response on the Interpretation of Higher Nervous Activity, and the Importance of the Ichthyopsid Type in This Respect

Pavlov, although rejecting the commonly accepted theory of cortical analyzers, thought that the cerebral cortex was the essential organ for the establishment and maintenance of conditioned reflexes and that these were the basis of learning and intelligent behavior. Both Bechtereff and Pavlov found that extirpation of the cortex in dogs removed the ability to form even simple associations, without affecting innate movements, but that puppies with only one hemisphere could be reared successfully and were apparently normal in behavior. In man there are many examples of recovery from the effects of brain injury which are hard to fit into the idea of cortical localization. Lashley destroyed the cortex of white rats to varying degrees and related this to their ability, before and after extirpation, to learn several kinds of mazes. He found that this ability depended not on the site, but on the degree of injury; and that complete cortex removal did not affect seriously either simple sensory discriminations or the ability to form simple associations. Buytendijk confirmed these findings, though disagreeing with Lashley on interpretation. Among the fishes, only the Dipnoi have a marked cortical region in the cerebral hemispheres; it is absent from both the teleosts and elasmobranchs. But in the teleosts certainly (the elasmobranchs have not been studied) many of the functions ascribed by Pavlov to the cerebral cortex are carried on without it. Teleosts form complex and simple conditioned motor responses with facility, including the types called by Pavlov (in the words of his translators) simultaneous, delayed, and trace conditioned reflexes. They show experimental disappearance of conditioned reflexes corresponding to the types of inhibition which he calls (a) external and (b) three kinds of internal inhibition: experimental extinction, conditioned inhibition, and differential inhibition. It is not necessary for our purpose to discuss the suitability of these terms in this context. It is, however, the last of these which constitutes for Pavlov the main central mechanism of sensory discrimination and is therefore linked intimately with the whole question of the site of sensory analysis and cerebral localization. Fishes, however, do not show "inhibition of delay." This is an essential feature in Pavlov's interpretation of those aspects of memory and intelligent behavior which are usually thought of as being above the simple habit formation level. The absence of this ability in fishes is the one important difference from dogs which may perhaps be related to the absence of a cortex. The level of normal adult teleostean behavior may be said roughly to equal that of decorticate white rats but to be superior to that of similarly treated dogs or man. An over-all comparison of the experimental evaluation of the behavior of these three vertebrate types suggests that the cortex has taken over and elaborated from subcortical centers in the thalamus and mid-brain an at present unknown kind of functional control of higher behavior whose importance increases in rather more than a linear relation to its structural increase in either size or complexity. Simple visual conditioning was shown by Sanders (1940) to be mediated by the tectum opticum and olfactory learning by the forebrain; but second-order learning was not retained after removal of the optic lobes. See also Neurath (1949), Nolte (1932), Sears (1934) and Sugi and Nomura (1937). The Ichthyopsid type is thus seen to be a stimulating and suggestive one in the study of behavior and of its underlying basis, not only because of the relatively simple brain pattern, but also because of the very wide range of sensory equipment and its central expression in brain form.

### C. General Principles of Conditioned Response Formation in Fishes

Conditioned responses in fishes are built up after a very variable number of presentations. This is rarely less than 10 or more than 80, and is more generally about 30. Normal behavior traits have a strong controlling influence (Bull, 1935a). If no response occurs after 200 reinforced presentations of the conditioned stimulus it is presumed that conditioning with that stimulus under the particular circumstances is not possible. The reason for this may lie in real absence of perception, or an over-riding inhibitory behavior trait, or the absence of suitable central connections. Once formed, the stability and experimental extinction of a conditioned response in a fish follows closely the general pattern found by Pavlov in dogs. In some instances they have survived many weeks' absence from experimental conditions.

Secondary (or second-order) conditioned reflexes are known to occur (Sanders, 1940) but have been little studied, and conditioned reflexes of higher order not at all.

Generalization takes place initially. Related stimuli, though differing perhaps both qualitatively and quantitatively, also evoke the response. This principle is used in two ways in studying sensory discrimination and perception. By continuing to reinforce all qualitatively similar stimuli, e.g. a salinity change, but reducing their quantitative values at successive though irregular presentations, a point is reached eventually when the impact of these stimuli on the normal environment fails to evoke a conditioned response. This lowest point of quantitative difference is taken to be the limit of sensory discrimination for that stimulus. Qualitative discrimination is found by reinforcing the initial conditioning stimulus and not reinforcing the contrasting one.

Conditioning to more than one stimulus over a given period is possible and also much used in discrimination studies; usually in the form of associating one stimulus with food and the other with shock.

Group conditioning is more rapid than individual.

## II. EXPERIMENTAL METHODS USED IN THE FORMATION OF CONDITIONED RESPONSES IN FISHES

### A. Apparatus

If an electric shock, or other kind of physical disturbance, e.g. touching with a rod, is used to elicit the registrable unconditioned motor response, the fish may be left free to move within the confines of a small tank but be suspended from a Marie tambour with a hook through the dorsal fin (or other suitable fulcrum) and the whole movement recorded by lever on a drum (Froloff, 1925). Instead of this suspension a record may just as readily be made of the disturbance of the surface of the water caused by the movements of the fish. This is done with a greased cover glass attached by a thread to a light lever (Bull, 1928, 1952).

Shock is given by means of an induction coil using stainless steel electrodes or agar bridges and nonpolarizable electrodes as devised by Lillie (see Bull, 1928). The electrodes occupy the whole area of one end of the trough or tank. A balance can be struck between the depth of water, strength of current, and size of fish, to ensure that the shock is just sufficient to cause a registrable response and is constant and reproducible. An electric shock may be unsuitable as an unconditioned stimulus (Sears, 1934; Ufland, 1929).

A more satisfactory, and natural, basis for conditioning is an unconditioned food-getting response; this can be specially adapted to meet the needs of the particular fish being studied. It must be an unequivocal specific directional movement in response to food after it has been perceived; a movement not normally performed by the fish, or only made with difficulty or effort in the circumstances of the experiment. Two broad types are possible. If the fish is by nature a free-swimming, active species, it can be conditioned to make an entry into a restricted space or compartment (Bull, 1928, et seq.) or to swim from conditions of comfort and relative freedom to a particular place where movement is restricted or from one place to another by a detour, round or against some obstacle. It has been found a great convenience to use an L-shaped tank inclined upwards away from the short limb. Fishes placed in such a tank take up residence in the deeper water in the corner formed by the short end and the arrangement is one which permits a variety of stimuli to be given. It has been said that what the maze was to Small, the inclined plane to Crozier, and the problem box to Thorndike, so the L-shaped tank is to Bull.

The devising of suitable methods for giving the conditioning stimulus offers the widest possible scope for experimental design. Experiments on sensory discrimination especially demand a high degree of inventiveness to ensure that the stimulus is acting alone in its pure state and that there is no concomitant factor giving rise to a response and a fallacious conclusion. For these reasons it is very desirable that the work should be done in a special building or place, preferably sound insulated, but at least isolated from the normal disturbances of a laboratory (Podkopaew, 1926; Bull, 1934). The presentation of the conditioning stimulus almost necessarily involves manipulations which could themselves have perceptual value. The manipulative part of the system should not therefore be in direct physical continuity with that part of the system actually housing the fish. An example of how this may be achieved is shown in Fig. 1A, B, illustrating the layout of an experiment in salinity discrimination. The fish must not be able to see the observer or detect his movements directly or indirectly. He and the manipulative parts of the system are thus separated by opaque screens from the fish housing part. Feeding tubes, stimulus-conveying devices, recording wires, etc., are all led through the screens. Visual observation is made indirectly with periscopes with high-grade optical fittings and shaded, focusing eyepieces. The space within the screens is kept brightly illuminated, and that without as dark as possible.

The successful provision of these conditions is one of ingenuity rather than actual difficulty, but depends partly on the kinds of fish used and partly on the facilities of the laboratory. For the larger and more important food fishes, whether marine or freshwater, the full resources of a large installation, with ample supplies of circulating water, pumping equipment, compressed air, etc., and privacy are essential. But valuable work can be done with both small tropical and temperate freshwater fish, with the minimum of space and equipment. For example, the minnow, *Phoxinus*, has been the most widely used and probably the most extensively conditioned of all fish, and much more fundamental work remains to be done.

### B. Procedure

In detail, this differs greatly from one stimulus to another, and in general, that for conditioning to shock from that to food. The principle is, however, to follow a rigid synchrony of conditioned and unconditioned stimuli on the lines already described. For experiments involving an electric shock the fish may be allowed to live a normal aquarium life, being taken from these conditions and put into the experimental system one-half hour before a sequence of tests. Then 5–10 conditioning presentations are carried out at about 5-minute intervals; after a further 30 minutes the fish is returned to its aquarium tank. This is repeated daily. Where food is used as the unconditioned stimulus, the fish generally lives in the experimental system for the duration of the experiment, but the same basic routine is followed. If a special building is used, the observer enters the building and leaves it at an interval of one-half hour from the sequence of tests or from any particular test.

Fish carrying eggs or young, or suffering from injury, or shock are unsuitable subjects.

### C. Representation and Interpretation of Results

Whatever form of physical recording is adopted, the actual progress of conditioning is best recorded by keeping a daily log giving a full statement of all operations and including a "freely subjective" account of the behavior of the subject. This is because the conditioned response is rarely identical with the unconditioned response it superficially resembles; it may perhaps progress only so far as "anticipatory" movements (though these may be very striking), or only half-completed locomotory actions, or go beyond the full reaction to a super-excitatory state, and even into a hysteria. The appearance of any part of the response is taken to be evidence of positive conditioning.

## III. SENSORY INVESTIGATIONS BY CONDITIONED RESPONSE TECHNIQUES

A fuller statement of sensory capacities is given elsewhere in the appropriate chapters (see especially Chap. II); the few examples given here are primarily chosen to illustrate the wide range covered by conditioned response and allied techniques and to give an indication of their value in this field of investigation.

FIG. 1. A: Plan of arrangement of apparatus in experiments on conditioned responses to salinity changes (right-hand side of diagram reduced 1.6 times more than left-hand side). From *Rept. Dove Marine Lab.* [3] **5**, 24. B: Elevation of portion of (A). One discharge and its associated tank only is shown. The reservoirs are at right angles. The distance between the reservoirs and experimental tank is much shortened both horizontally and vertically. The separating screens and many supply tubes are also omitted. From *Rept. Dove Marine Lab.* [3] **5**, 24. A, reservoir for stimulus water. B, normal supply (also circulates through C), flows from a–e and screen S to tanks 1–5. Before entering each tank, flow is "broken" by constant-level device D, and then through Davies' double-surface condensers through which sea water supply to whole system also passes, thus ensuring complete temperature equality. Tanks slope upwards from R to P at 1 in 30, fishes live below E at R, food chambers at X, periscopes, P. T, overflow below P.

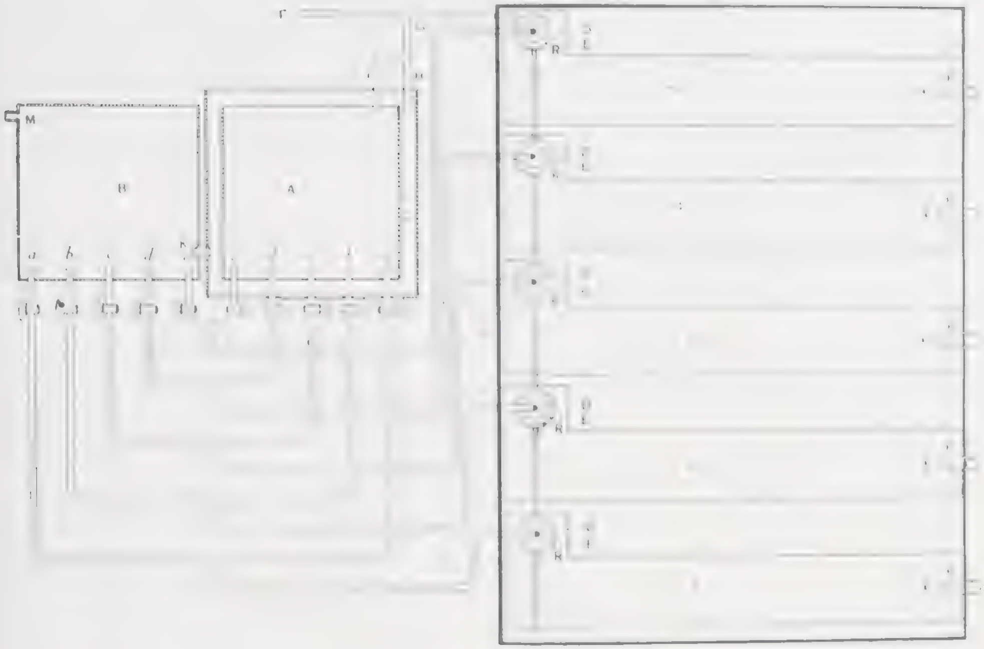

Fig. 1A

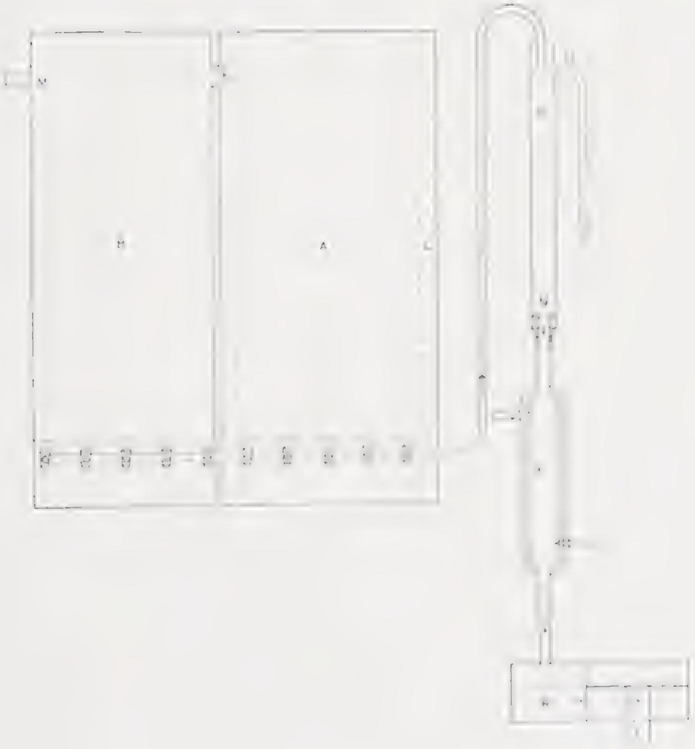

Fig. 1B

### A. Sight

Froloff, Bull, and others have established true conditioned responses in a wide range of fishes to a number of visual stimuli, using an electric shock as the unconditioned stimulus. *Blennius pholis* has been shown by the same technique followed by differential conditioning to have a comprehensive capacity for wavelength discrimination, using Wratten light filters of high purity and known characteristics. This is so in dark-adapted as well as in light-adapted fish.

The more usual form of visual conditioning experiment, however—and one which is readily adapted to class demonstration—uses as its basis the entry of the fish into a food chamber, bottle, or space, or swimming to a special receptacle, scoop, shelf, or hook, to take food presented there. When trained to one visual stimulus, a contrasting visual signal is associated either with punishment or nonreward, or with the execution of another similar response with different spatial relationships. These also have shown that fishes have a wide range of visual achievement. They form conditioned responses to, and discriminate between a considerable range of colors, brightness, form, size, number, and position of objects. These include the ability to distinguish between pieces of cardboard bearing the letters U and E, W and L, etc., as well as between groups of two or four letters (Fisher, 1941). For a comprehensive account of visual learning see Herter (1953). See also Chap. II: Part I.

### B. Sound

It has now become accepted that, to arrive at a reliable conclusion on the sense of hearing in intact fishes, a conditioned response technique is almost essential. Without it, and prior to its introduction and use in the study of fishes by Miss McDonald (1922), there was much disagreement on the ability of fishes to hear (see Chap. II: Part 2). With the exception of the recent work of Kleerekoper and Chagnon (1954) most of the conditioned auditory studies of fish have stemmed from von Frisch and his colleagues. A comprehensive range of genera and species have now been shown to have the ability to form responses to a variety of tones between 16 and 7000 c.p.s.; to distinguish by differential conditioning intervals of from a quarter-tone to one and a third octaves at a number of different ranges; and to recognize the sources of two sounds. Any disagreements that remain on the general problem relate to the purely physical difficulties of precisely measuring and presenting the vibratory stimuli and giving them a proper form in terms of frequency, intensity, and location. Herter (1953) reports that he has extended von Frisch's simple experiment with *Ameiurus*, in which the fish was trained to associate the whistle of the experimenter with food, to showing that it can also be conditioned to human vocal utterances even to the extent of responding to, and distinguishing between the spoken words "Adam" and "Eve"!

### C. Smell and Taste

Hasler (1954) has recently reviewed olfaction in fishes and rightly says that the role of the sense of smell in fishes is a "subtle" and important one (see also Chap. II: Part 3). He has himself made use of the conditioned response technique both experimentally and in discussion of fishery problems.

Göz (1941), by conditioning to food, and a light blow from a glass rod, found that *Phoxinus* discriminated olfactorily between many species of fish and between individuals of its own kind. Walker and Hasler (1949) were able to condition small groups of blinded blunt-nosed minnows (*Hyborhynchus notatus*) to water bearing the odors of aquatic plants, obtained merely by rinsing the plants in the water, and to show that they discriminated between "pairs" of odors, e.g. *Myriophyllum exalbescens* and *Ceratophyllum demersum*, when one was followed by food and the other by shock. The biological assay of pollutants is possible by the same procedure. Hasler and Wisby (1949) showed discrimination in *Hyborhynchus notatus* for phenol and *p*-chlorophenol at dilutions of less than $5 \times 10^{-3}$ p.p.m. *Phoxinus* has been conditioned to eugenol at dilutions of $17 \times 10^{-6}$ p.p.m. and to phenylethyl alcohol at $23 \times 10^{-6}$ p.p.m. and can distinguish between these odors at these dilutions. Removal of the olfactory lobes destroys this ability (Neurath, 1949).

*Gustatory* stimuli are also important natural stimuli and readily evoke conditioned responses, but few species have been studied.

*Blennius pholis* has been conditioned to as little as 0.00075% (wet weight) extract of *Mytilus*, and *Blennius gattorugine* to 0.01% similar extracts of *Nereis diversicolor* (Bull, 1930).

*Phoxinus* has been more extensively used with the four recognized categories of taste, e.g. salt, sour, sweet, bitter (Trudel, 1929; Strieck, 1924; Krinner, 1935). It has been conditioned to salt, NaCl, (0.00023%), acetic acid (0.15%), saccharose (0.00072%), and quinine (0.0025%). Differential conditioning has established an ability to discriminate between a number of natural sugars and these from saccharin (Trudel, 1929).

### D. Current Flow

Fishes appear to be unable to form conditioned responses to changes in rates of current flow where these are of the order of between 5 and 40 cm. per minute. This was my conclusion after 2 years' work on 40 species of fish, both sedentary and free swimming. It supports the findings of Gray and others that fish give no response to rectilineal acceleration except when this is accompanied by visual stimulation. Higher speeds have not been studied.

A change in direction of current flow of 90° or more, but not less, has perceptual significance for, and has been used as a conditioning stimulus, in the sedentary fish *Blennius gattorugine* (Bull, 1935c). This is an absolute sense of direction for it is not dependent on the orientation of the fish to the current.

### E. Hydrogen Ion Concentration

Only Bull (1940) has reported on the ability of fishes to perceive changes in pH as shown by the conditioned response method. His experiments were confined to marine fishes. Initial conditioning took place readily to a change in pH from 7.95–8.22 (the range for the sea water at Cullercoats where the work was done) to approximately 7.0, brought about by adding a small quantity of $CO_2$-saturated sea water to a much larger quantity of normal sea water as it flowed past the fish. After establishment of the response, the generalization procedure was used to find the minimum perceivable change. Forty specimens of fishes belonging to 20 species and including the genera *Anguilla*, *Nerophis*, *Gadus*, *Onos*, *Crenilabrus*, *Gobius*, *Blennius*, *Chirolophis*, *Centronotus*, *Zoarces*, *Cottus*, *Gasterosteus*, *Spinachia*, *Rhombus*, and *Pleuronectes* formed conditioned motor responses to a reduction in pH of 0.04–0.10.

### F. Salinity

As a conditioning stimulus for marine fishes this is a striking and important one. A similar range of individuals and species as was used for pH discrimination was also used by Bull (1938) in his study of this sense. They were all found able to note a sharp change of from 34 ‰ to 30 ‰ as a conditioning signal, and later to discriminate between changes of 0.5 ‰ or less. *Gobius flavescens* is outstanding in being able to perceive differences of 0.06 ‰.

### G. Temperature

Gradient tank experiments have perhaps been seriously misleading as to the perception of temperature changes by fishes. The freshwater fishes, *Phoxinus*, *Nemachilus*, *Misgurnus*, and *Ameiurus* have been shown by Dijkgraaf (1940) to be able to form conditioned responses to both temperature rises and falls of 0.5° C. acting momentarily. Bull has shown (1928, 1936) in marine fishes that there is a widespread ability to detect and use as conditioning stimuli very small increases in water temperature. Table I summarizes his findings. In all cases, initial conditioning was to a comparatively large change; the final limits of perception were obtained by the generalization procedure.

TABLE I
SUMMARY OF EXPERIMENTS ON FORMATION OF CONDITIONED RESPONSES IN FISHES TO A RISE IN TEMPERATURE[a]

| Species | Duration of Experiment | Number of Associations Required for Production of Conditioned Response to Thermal Increase | Total Number of Associations in Experiment | Minimum Effective Thermal Conditioned Stimulus (° C.) |
|---|---|---|---|---|
| *Spinachia vulgaris* Flem. | 6.x.33–15.xii.33 | 41 | 84 | 0·05 |
| *Nerophis lumbriciformis* (Pennant) | 10.iii.34–28.v.34 | 56 | 76 | 0·07 |
| *Gadus callarias* L. | 12.v.32–19.vii.32 | 18 | 61 | 0·05 |
| *Gadus merlangus* L. | 30.v.32–24.ix.32 | 10 | 73 | 0·03 |
| *Gadus virens* L. | 6.x.33–15.xii.33 | 24 | 83 | 0·06 |
| *Onos mustela* (L.) | 10.iii.34–17.vi.34 | 12 | 29 | 0·03 |
| *Raniceps raninus* (L.) | 11.xi.32–8.vi.33 | 31 | 147 | 0·05 |
| *Blennius pholis* L. | 10.iii.34–28.v.34 | 33 | 95 | 0·03 |
| *Chirolophis galerita* (L.) | 18.iv.34–9.v.34 | 25 | 33 | — |
| *Centronotus gunnellus* (L.) | 10.iii.34–28.v.34 | 39 | 95 | 0·03 |
| *Zoarces viviparus* (L.) | 10.iii.34–28.v.34 | 25 | 95 | 0·03 |
| *Gobius flavescens* Fab. | 6.x.33–15.xii.33 | 14 | 77 | 0·05 |
| *Platichthys flesus* (L.) | 10.viii.33–5.x.33 | 20 | 59 | 0·05 |
| *Pleuronectes platessa* L. | 24.viii.32–25.x.32 | 11 | 71 | 0·06 |
|  | 6.x.33–15.xii.33 | 21 | 78 | 0·05 |
| *Cottus scorpius* L. | 26.vi.33–7.xi.33 | 12 | 88 | 0·05 |
| *Cottus bubalis* Euphrasén | 10.iii.34–28.v.34 | 27 | 108 | 0·03 |
| *Cyclopterus lumpus* L. | 6.x.33–15.xii.33 | 32 | 67 | 0·05 |
| *Liparis montagui* (Don.) | 6.x.33–15.xii.33 | 26 | 71 | 0·03 |
| *Crenilabrus melops* (L.) | 6.x.33–15.xii.33 | 29 | 71 | 0·05 |

[a] From *J. Marine Biol. Assoc. United Kingdom* [n.s.] **21,** 24.

### H. Pressure

Published observations on conditioning to pressure changes are confined to those of Dijkgraaf (1941a, b; 1942), using a simple manometric device to produce the pressure signal, and food as the unconditioned stimulus, and the "food-seeking" reaction as the registrable response. In these experiments, 10–40 associated presentations of a change of 20 cm. in manometer height were enough to produce the conditioned response in blinded *Phoxinus*. By generalization he then showed perceptual ability for decreases in pressure of 0.5 cm. and increases of 1.0 cm.

## IV. THE RELATION OF THE LABORATORY STUDY OF CONDITIONED RESPONSES TO BEHAVIOR IN NORMAL ENVIRONMENTS, AND ITS VALUE IN FISHERY INVESTIGATIONS

Hasler (1954) and Bull (1930) have both discussed major migratory movements of fish in terms of conditioned response theory. Normal behavior traits also profoundly influence capacity to form conditioned responses in the laboratory (Bull, 1935b).

The wide practical application of the conditioned response in human affairs, within the spheres of individual training, education, medicine, political indoctrination, and warfare, leave no room for doubt that where its use is indicated to solve some practical problem in the lower vertebrates it should be given serious large-scale trial. One such suggestion (Hasler, 1954) is that morpholine (which is detectable by salmon at dilutions of $1 \times 10^{-6}$ p.p.m., but is neither attractant nor repellent) be used to decoy salmon on their return migration to a stream other than that from which they originated, by previously conditioning them, as fry and fingerlings, to its smell. Conversely, there is a possibility that large-scale conditioning involving the linking of "near-capture" with a preceding, regularly coincident perceptual stimulus, e.g. the sound of echo-sounders with trawling, may lead eventually to a decrease in fish captures on a commercial scale. The determination of the minimal perceptual values of such potentially important hindrances to commercial fishing is made possible by this very specialized field of study.

## REFERENCES

Russell and Bull (1932) give a full bibliography of relevant references up to 1931; these references are listed here only if quoted in the text. References are also included here to a number of works since 1931 which are not quoted in the text but are concerned with this aspect of fish behavior.

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## Chapter III: Part 2

# BEHAVIOR:
# THE ETHOLOGICAL ANALYSIS OF FISH BEHAVIOR

G. P. Baerends

*Page*

I. Introduction . . . . . . . . . . . . . . . . . . . . . . . 229
II. Coordinating Mechanisms . . . . . . . . . . . . . . . . . . 231
  A. The Fixed Pattern and its Orienting Component (Taxis) . . . . 231
  B. Comparison of the Fixed Pattern with Elements of "Lower" Integration 236
  C. Integrative Mechanisms of Levels Higher than the Instinctive Activity 240
    1. Instincts and the Hierarchical Organization of Behavior . . . . 240
    2. Interaction of Instincts . . . . . . . . . . . . . . 246
    3. Appetitive Behavior and the Consummatory Act . . . . . . 248
  D. Conclusion . . . . . . . . . . . . . . . . . . . . . 251
III. Releasing Mechanisms . . . . . . . . . . . . . . . . . . 252
  A. Sign Stimuli . . . . . . . . . . . . . . . . . . . . 252
  B. The Evaluation of Stimuli . . . . . . . . . . . . . . . 253
  C. Heterogeneous Summation . . . . . . . . . . . . . . . 254
  D. Definition . . . . . . . . . . . . . . . . . . . . . 255
  E. Supernormal Stimuli . . . . . . . . . . . . . . . . . 255
  F. The Ontogeny of Releasing Mechanisms . . . . . . . . . . 255
  G. Releasing and Directing Stimuli . . . . . . . . . . . . 256
IV. The Interaction between Internal and External Factors . . . . . 258
  A. The Causation of "Drive" . . . . . . . . . . . . . . . 258
  B. The Influence of Internal Factors on the Evaluation of a Stimulus . 259
  C. The Influence of Internal Factors on Orienting Reflexes . . . . 260
  D. Adaptation, Extinction . . . . . . . . . . . . . . . . 260
V. Ritualization . . . . . . . . . . . . . . . . . . . . . 261
  A. Social Releasers . . . . . . . . . . . . . . . . . . 261
  B. The Derivation of Social Releasers . . . . . . . . . . . 262
  References . . . . . . . . . . . . . . . . . . . . . 265

## I. INTRODUCTION

The aim of ethology is to study behavior with objective methods primarily through causal analysis. Though not denying the existence of subjective feelings in animals, the ethologist is of the opinion that these phenomena are beyond our possibility of knowledge and therefore cannot be brought into this analysis. In this he differs from some schools of animal psychology, particularly in Europe, which believe that it is absolutely essential for the study of behavior to take into consideration the subjective feelings, which are then often regarded as causes of behavior.

The ethologist differs chiefly from the attitude of the American comparative psychologists in that he is primarily interested in analyzing and understanding the entire natural behavior repertoire of the animal, whereas the comparative psychologist, being primarily interested in the mechanisms of learning as such, has not bothered too much about studying the animal under natural conditions. This not very essential difference has wrongly led the ethologists to lay too much emphasis on the innate aspects of behavior, and some experimental psychologists (Lehrman, 1953) to over-accentuate learning and in general the role of the environment, while in fact we have to realize that we should in every instance specify in detail how each aspect of a behavior develops.

As is usual in biology, ethology has three main problems. Firstly, to describe behavior and to understand the underlying physiological mechanisms. Secondly, in cooperation with ecology, to study the biological significance of the different behavior patterns. Thirdly, to understand the development of behavior in the course of evolution. In this book we shall be mainly concerned with the physiological aspects of ethology. We shall try to build up a picture of the structure of behavior in fishes, giving at the same time an idea of the data on which the conclusions are based. It must be realized that this picture is very tentative and is, therefore, primarily meant as a working hypothesis to support further research. Any hypothesis has to be checked continuously and amended or changed every time this seems necessary. Facts should be collected to improve the hypothesis, never only to prove it.

Various descriptions of fish behavior have already been published. We will mention here the work on the three-spined stickleback (*Gasterosteus aculeatus*) (Leiner, 1929, 1930; Wunder, 1930; ter Pelkwijk and Tinbergen, 1937; Tinbergen, 1951, 1953a; van Iersel, 1953), on the cichlid fishes (Breder, 1934; Peters, 1937, 1941; Seitz, 1940, 1942, 1948; Noble and Curtis, 1939; Aronson, 1945, 1949; Baerends and Baerends-Van Roon, 1950), on centrarchids (Breder, 1936; Greenberg, 1947), on cyprinodontids (Clark and Aronson, 1951; Clark, Aronson, and Gordon, 1954, Baerends, Brouwer, and Waterbolk, 1955), on gobiids (Nyman, 1953, Tavolga, 1954), on the bullhead, *Cottus gobio* (Morris, 1954), on the labyrinth fish, *Betta splendens* (Lissmann, 1933), and on the catfish, *Ameiurus nebulosus* (Breder, 1935).

Most of these publications are chiefly concerned with reproductive behavior. They describe, in greater or less detail, how, by a series of ceremonial activities, for instance, a male stickleback leads the female towards the nest, how a male bitterling leads its partner to a mussel to deposit eggs, how further rival males establish and defend territories, how in some groups care is taken of the young and how a stickleback builds its elaborate nest from water plants.

## II. COORDINATING MECHANISMS

### A. The Fixed Pattern and its Orienting Component (Taxis)

As behavior is built up of movements of muscles the most objective description of the performances mentioned above would be one in terms of muscle contractions and relaxations. Nevertheless, none of the authors mentioned has ever attempted this; they have used more complex units in which many different muscles are involved, activities like yawning, eating algae, digging sand, leading a female, fanning eggs, calling young, etc. To every student of behavior it appears self-evident and natural to describe behavior in terms of activities of this order. Although different activities may, to some extent, use the same muscle or muscle groups, the pattern in which the component muscle contractions and relaxations are coordinated is very characteristic.

Activities of this kind are typical for each species; they have a very limited variability and can, therefore, always be recognized, even if they occur under abnormal circumstances in which their normal function is impeded or lost. Though individuals may show persistent differences in the performance of these activities, those differences are always slight and usually only become obvious after much experience in observing the species concerned. Usually we find a similar activity also in related species, but then often in specifically different modifications. These behavior patterns develop in each individual of a species in the same way and with the same final result, even if the fish are reared from the earliest stage in complete isolation, e.g. a male *Lebistes reticulatus* kept alone from the moment it was born, shows all activities of the courtship ceremony when, after maturation, it is confronted with a female. This means that genetic factors determine the form of these activities in the same way as they determine the development and final form of organs. Learning, from observing the behavior of another member of the species, cannot be shown to play a role here.

Because of all these characteristics Whitman (1898), Heinroth (1910), and particularly Lorenz (1935, 1937a, b, 1939, 1952) have emphasized that these stereotyped activities are just as typical for a species as morphological structures and, therefore, can be used as taxonomic features. Moreover, they realized that the concept of homology can be applied to these activities and so they founded a truly comparative study of behavior. Our first task must be now to define these stereotyped activities more exactly, to study their properties and to see how strictly they are distinguishable from other behavior elements.

Lorenz (1937b) discriminates sharply between two types of coordinated muscle activity. In one type, the coordination is fixed; an external stimulus is usually necessary to release or to maintain the activity. The strength of this stimulus influences the intensity of the activity (e.g. the number of muscles involved), but it has no other effects on its form ("Bewegungsnorm").

In the other type, the coordination is less rigid; here adaptive changes corresponding to properties of the stimulus are possible in accordance with fixed rules ("Reaktionsnorm").

In fact, whereas this second type cannot exist without the external situation, the first type is much less dependent on it. As a rule both types occur together, like a propelling and a steering mechanism, as was first experimentally shown by Lorenz and Tinbergen (1938) in their study on the egg rolling activity of the grey lag-goose.

In fishes this principle can be illustrated by studying the movement by means of which the male three-spined stickleback (*Gasterosteus aculeatus*) ventilates the eggs in its nest (Tinbergen, 1951; van Iersel, 1953; unpublished data by I. Kristensen and E. Arendsen de Wolf-Exalto). Figure 1A shows the male when fanning. By bringing potassium permanganate crystals near the fish it can be established that the pectoral fins exert a forward pressure on the water and so direct a current against and through the nest. At the same time the tail and the caudal fin exert a backward pressure that is equal and opposite to the forward pressure of the pectorals and thereby keeps the fish on the spot. This typical coordination between the pectorals and the tail is the characteristic "fixed component" of the activity. For its release, the presence of a nest with fertilized eggs is usually necessary but it may sometimes occur in the absence of the adequate external situation (as a vacuum activity, see below). Then its coordination is still the same so that it is easily recognizable. In this abnormal case the longitudinal axis of the fish is held horizontal whereas, when it is fanning a nest, it keeps a definite angle to the horizontal plane in which the nest usually is. When the nest was tilted, the fish tended to keep the angle between the longitudinal axes of its body and of the nest constant (Fig. 1B). Meanwhile its sagittal plane remained vertical; as von Holst (1950a, b) has shown, this latter orientation is provided for by light and gravity stimuli. By displacing the nest in the horizontal plane it was proved that the exact location of the fish during fanning is determined by optical beacons, such as the entrance of the nest and even more by other objects in the environment, e.g. the corner of the tank (Fig. 1C).

Therefore, in contrast to the coordinations of the fixed component producing the aerating current, the coordinations orienting the animal in space during the action are continuously influenced by external stimuli. This supports Lorenz' statement that the activities we have used as units for description consist of two principally different parts: an invariable pattern of muscle activity on which is superimposed another pattern, variable in relation to external stimulation.

As the invariable part seems to be typical for behavior that is usually —in a loose sense—attributed to "instinct." Lorenz (1937a, b) named it "instinctive activity" or "instinctive movement" ("Instinkthandlung," "Instinktbewegung"). He also used the term "Erbkoordination" while in

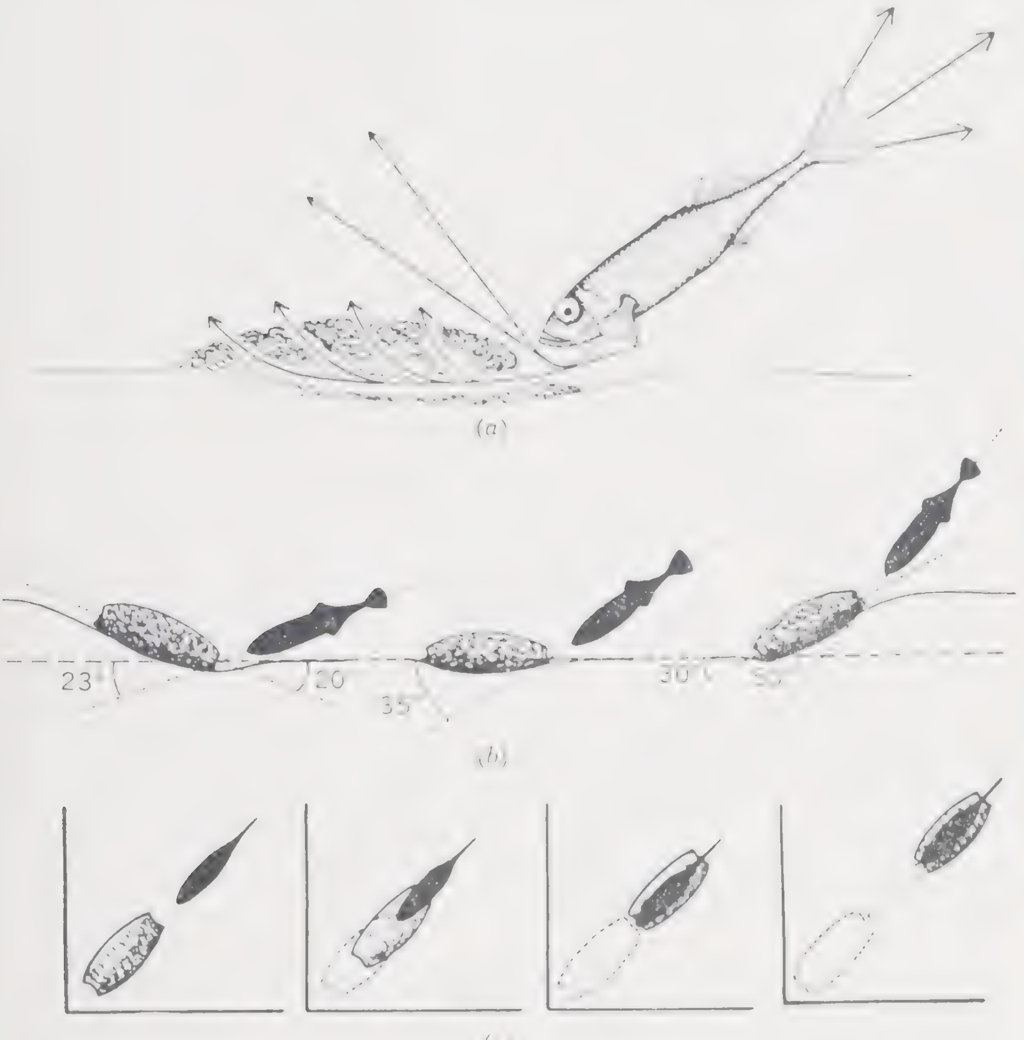

FIG. 1. Fanning in the three-spined stickleback. A: The direction of the currents aroused (from Tinbergen, 1951). B: The influence of tilting the nest on the orientation during fanning. C: Experiment on the relative importance of the nest and of other environmental beacons (the corner of the tank) on the position of the fish during fanning.

the English language the term "fixed (action) pattern" was introduced (Thorpe, 1951). Later "Instinkthandlung" (instinctive activity) was used by several authors for the combination of propelling and steering activity and "Instinktbewegung" (instinctive movement) for the former part only. For the steering component the word "taxis" is used and, as Koehler (1950) has emphasized, it should be restricted to the orienting mechanism only and not, as is often done, also used for an entire oriented locomotion. In the following we will use the terms "fixed pattern," "taxis," and, for both in combination, "instinctive activity" ("Instinkt-Taxis Verschränkung").

That it is desirable to have a term for the combined activities can be illustrated by the following example, taken from a study of the courtship of the male *Lebistes reticulatus* (Baerends, Brouwer, and Waterbolk, 1955). In this courtship two phases can be distinguished. First, in the "leading" phase the male tries to lure the female away from the school of guppies. If this succeeds, the "checking" phase follows in which the male stops a further forward movement of the female and finally attempts to copulate. Both in "leading" and "checking," a sigmoid posture, combined with a back and forth swimming movement is performed in front of the female. On closer analysis, this sigmoid appears to be the same fixed pattern in both phases. However, in leading it is combined with a set of taxis components directing the sigmoid away from the female and in checking with a set of components orienting the activity perpendicularly towards the direction of the advancing female.

Lorenz's concept of the "fixed pattern" has become a basis on which the different branches of ethology have built up their hypotheses. Apart from the already mentioned facts (1) that the coordination pattern is fixed and stereotyped and (2) that as a rule it occurs only when released by a specific external stimulus situation, Lorenz has described two other characteristics that should particularly distinguish this element from the "reflexes."

Firstly, under completely constant external conditions, the occurrence and the intensity of the activity fluctuate with time. This is illustrated by Fig. 2 which shows the number of seconds a male three-spined stickleback spends in fanning during each of 24 consecutive 5-minute periods when caring for its eggs (van Iersel, 1953). Secondly, as a rule the intensity of an activity released by a given stimulus increases with the lapse of time since its previous occurrence. When van Iersel prevented the stickleback from fanning its nest for several minutes by scaring the fish mildly, he found a rise above the mean of the fanning activity after the fish had returned to the nest. The influence of the accumulation of carbon dioxide in the nest as an external factor was excluded from these experiments by keeping the nests completely covered with a watch glass. Conversely, the longer the period since the activity was last performed the weaker the stimulus needed to release a definite intensity stage (threshold lowering). For instance, Lorenz (personal communication) and Baerends and Baerends-van Roon (1950) have observed cichlid fishes, which had failed to breed successfully, guarding a flock of *Daphnia* as if they were their own young. ("Overflow" of an activity; reaction to suboptimal stimuli; Bastock, Morris, and Moynihan, 1953.) If an activity is not released for a very long time it may be performed without any of the adequate releasing stimuli being present. This Lorenz calls "vacuum activity" ("Leerlaufreaktion"). Thus, we have several times seen single *Lebistes* males, kept in complete isolation since birth, performing the typical sigmoid display while still alone. Van Iersel (1953) mentions that at the height of the period of parental care the male three-spined stickleback, when away from the nest, may suddenly start fanning *in vacuo* (in a horizontal position) and then swim towards the nest and resume ordinary fanning.

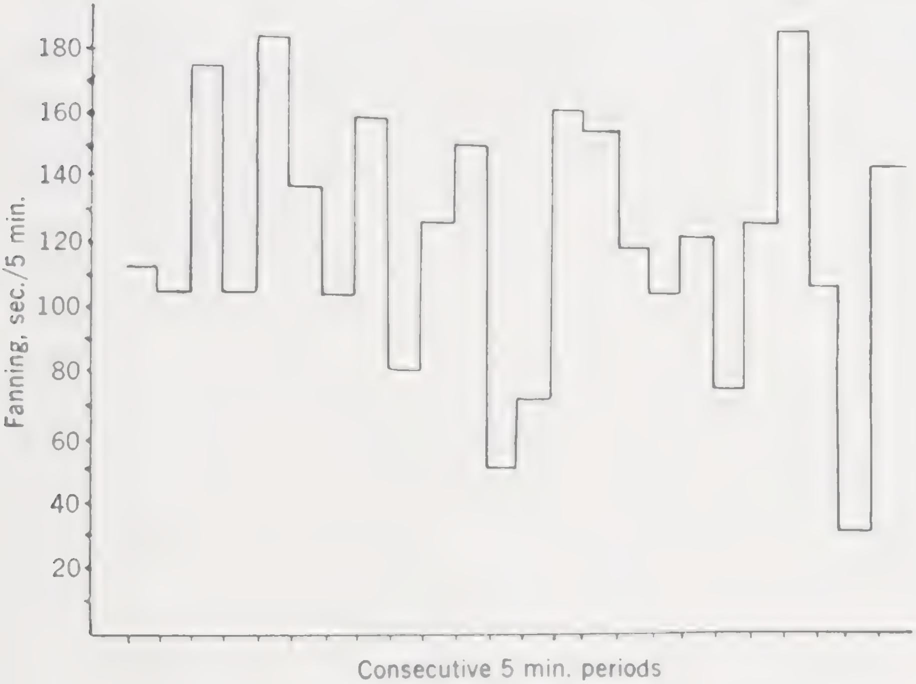

FIG. 2. Fluctuation in the fanning activity of a male three-spined stickleback (from van Iersel, 1953).

All these phenomena indicate that, besides the external releasing situation, an internal factor is of importance which can apparently be "accumulated" and "exhausted."

Secondly, Lorenz has stressed a fact already recognized by Craig (1918): the fixed pattern is preceded by "appetitive behavior," a searching for the stimulus that can release the activity. The performance of the activity (consummatory act in Craig's terminology) stops the appetitive behavior. This appetitive behavior can have a fixed form but usually it is variable and often includes elements in which learning and other "intelligent" processes play a role. A stickleback that has left its nest for a while shows this appetitive behavior when coming back to resume fanning. Appetitive behavior may even include attempts to pull off a watch glass which the experimenter has put on the nest.

Lorenz postulated that the internal factor underlying the occurrence of a fixed pattern also starts and maintains the appetitive behavior until the external releasing situation for the fixed pattern is found. To stress the difference between fixed pattern and reflex he emphasized that the animal never searches actively for the stimuli which would elicit its reflexes.

Thus, the internal factor, which seems to underlie the fixed pattern but not the reflex, might be a means of distinguishing between them. Lorenz himself has started checking this idea by comparing his ethological findings with investigations on "reflexes" at a purely physiological level, particularly those by Sherrington and his co-workers (1948) on mammals and by von Holst (1934a, b; 1935a; 1937a, b; 1939) on fishes. A convenient way to follow his example is to look at the movements during the fanning of stickleback eggs in more detail.

### B. Comparison of the Fixed Pattern with Elements of "Lower" Integration

We have mentioned that the core of the fanning pattern consists of a propelling movement of the tail and a forward movement of the pectorals. Both are locomotory movements normally used, respectively, in forward and in backward swimming, but here acting against each other and thus fixing the fish on the spot while producing the aerating current. Besides these locomotory movements there are the taxis components. We will consider both elements more closely.

The physiological basis of locomotory movements of fishes is discussed in Chap. I and brief comments only will be made here. Early theories (Friedlander, 1894; Philippson, 1905) held that rhythmical locomotory movements are maintained and coordinated by chains of reflexes activated by peripheral stimuli arising from mechanical deformations. These theories were modified when later work (Graham Brown, 1912a, b; von Holst, 1935a; Gray, 1936; Gray and Sand, 1936; ten Cate, 1940) showed that mechanisms inside the central nervous system could maintain coordinated movements when peripheral reflexes were experimentally excluded over part of the trunk.

Von Holst (1937b; 1939) analyzed in detail the pattern of coordination between fins and between the fin rays of one fin in several species of fishes. He postulated that complexes of mutually interacting cell mechanisms are present in the central nervous system and are responsible for its central coordinating activity. Two principles of interaction were established: (1) the magnet effect, when the frequency of the rhythm of one cell group may be enforced onto another, (2) the superposition effect, when the amplitude of the movements is the algebraic total of the undulations of the dominating and dependent rhythms.

Von Holst speaks of "absolute coordination" when the dominating rhythm gains complete control over the dependent rhythm. Since each mechanism has a stronger or weaker tendency to maintain its own frequency ("Beharrungstendenz"), the dominance is often incomplete. The dominating rhythm is then alternately more and less successful in imposing its rhythm and von Holst named this "relative coordination." This occurs particularly often in fish but can also be observed in other animals.

The idea of complexes of mutually interacting cell groups is an elegant explanation of the central coordinating activity of the spinal cord. We may expect it to underlie not only locomotory movements but probably also instinctive activities. In addition, differences in conductivity, in lengths of nervous pathways, and in threshold of synapses must contribute to the coordination of the activities of motor units in space and time.

Besides the problem of coordination, there is also the problem of initiation and maintenance of the movement. Von Holst believes that the central mechanisms causing locomotory coordination also produce rhythmical impulses "automatically," i.e., independently of *specific* afferent inflow, when the central excitatory state has reached a certain level. He compares these mechanisms with the respiratory center to which he thinks they may be closely related (von Holst, 1934b; le Mare, 1936). Other authors (ten Cate, 1940; Lissmann, 1946a, b) have questioned the automatic nature of rhythmically firing cell groups. They consider that the rhythm must be initiated by specific impulses from higher centers in the central nervous system or from peripheral receptors. Though Lissmann is convinced that waves do not spread over the body as a chain of central reflexes, he is inclined to consider the undulatory posture of the dogfish as one single reflex which can only move along the body if there is a rhythmical afferent input.

Some other arguments have been used to support the idea of an intrinsic factor in the causation of activities: instinctive activities often persist for some time after the external stimulation has stopped. A comparable phenomenon has been described by Sherrington *et al.* (see 1948) under the name after-discharge.

A relative change of frequency or intensity of a movement after it has been suppressed for some time (as we have found in a stickleback prevented from fanning) occurs also at lower levels of integration. Von Holst (1937b) in sea horses, and le Mare (1936) in dogfishes were able to inhibit the undulatory movement, respectively, in the dorsal fin and in the entire body by peripheral stimulation. After this stimulation had stopped, for some time the undulatory movement went on but at increased amplitude. The reverse also occurs: when von Holst (1934a) directed a current against the body of a goldfish in which the frontal part of the medulla was transected, the amplitude of the existing locomotory waves increased during and also for some time after the stimulation. Then, however, the amplitude decreased and was for a short period below the normal level before it reached the latter again. Similar phenomena have been described, for mammalian preparations, by Sherrington (1948) as "rebound" and "spinal contrast." Lorenz (1937a, b) considers that these phenomena provide a strong argument that there is an intrinsic factor, which can build up as well as subside, underlying this kind of activity. However, the same phenomena might also be explained by changes in excitatory state that need not be determined by endogenous factors but could be caused by peripheral stimuli (see Kennedy, 1954). Spinal contrast or rebound only occur after a stimulus has been applied, not after a period of rest.

Apart from this type of experiment we might be able to obtain information about the possible existence of autonomous intrinsic mechanisms: (1) by leading off action potentials from the central nervous system; and (2) by observations during ontogeny. The existence of rhythmical electrical activity in the central nervous system has been established in many cases. Adrian and Buytendijk (1931) found in a completely isolated brain of a goldfish a rhythm corresponding roughly with the usual respiratory rhythm of these fishes. This created general interest in intrinsic factors, which, however, has not yet led to more thorough research of this kind in fish.

Neither do we know of any ontogenetical evidence for the presence of intrinsic factors obtained from fishes, but Weiss's experiments in Amphibia may be mentioned here. Weiss (1941) implanted a piece of embryonic spinal cord and an embryonic leg rudiment into the connective tissue of an axolotl. Fibers grew from the implanted nervous tissue towards the implanted limb muscles but there was no nervous contact with the spinal cord of the host. Yet, as soon as motor fibers had made contact with the muscles, the limb spontaneously performed rhythmical movements before sensory connections had been completed.

From this survey we may conclude that there is strong evidence that the spinal cord contains mechanisms coordinating muscle activity. In a number of experiments it has been shown that afferent inflow is necessary to initiate and maintain persistent rhythmical coordinations, such as those occurring in locomotion. in some cases this inflow must be rhythmical though not quite specific, but in other cases very unspecific arhythmical stimuli are sufficient. Though it seems not unlikely that cells of nervous centers might produce impulses autonomously, no conclusive evidence is yet available on this point.

The output of these centrally coordinated mechanisms can be modulated by other coordinating mechanisms that are chiefly controlled peripherally. Reflexes of this kind have been extensively studied in fish (von Holst, 1934a, b; 1937a, von Holst and le Mare, 1935; Eberhard, Fabricius, and von Holst, 1939; le Mare, 1936, Lissmann, 1946a, b). In general we can say that external stimuli (particularly tactile ones, e.g. currents) are able to evoke special positions of the body and the fins, the character of which depends on the area stimulated. Many of these reflexes adapt the movements of the fish to changing hydrodynamical conditions. They differ chiefly from the central coordinations on which they are superimposed in that they need a constant input of specific external information. It is outside the scope of this chapter to discuss the mechanisms of these reflexes to which the orienting components (taxes) also belong and for which I can, for instance, refer to von Holst (1935b, 1950a).

Two facts suggest that these reflexes act on the central motor mechanisms peripherally from the central coordinating system: (1) peripheral stimulation of the dominant fin does not affect the rhythm of the dependent one; and (2) the rebound effect after peripheral stimulation (p. 238).

We started this survey of physiological evidence to see if there is a distinction between "instinctive activity" as defined at the beginning of this chapter and the lower levels of integration studied mainly in physiological preparations rather than in intact animals. Now our final conclusion must be that there is no sharp distinction. When breaking up the instinctive activities into ever smaller parts or lower levels of integration we find the same picture every time: a core of centrally coordinated muscle contractions and relaxations, initiated by more or less specific information or perhaps by automatic impulses produced in the coordinating nervous mechanisms, and this is oriented in the environment by other coordinated muscle activities which are superimposed on it and mainly differ from it in that they are continuously determined by specific peripheral stimuli.

Phenomena like inhibition, facilitation, changes of excitatory state, after-discharge, and rebound exist at all levels of integration from the simplest coordination to the most complicated activities.

## C. Integrative Mechanisms of Levels Higher than the Instinctive Activity

Having compared instinctive activities with elements of lower integration, we will now consider whether coordinations of higher level can also be distinguished.

### 1. Instincts and the Hierarchical Organization of Behavior

In many cichlid fishes (*Cichlasoma*, *Aequidens*, *Hemichromis*) at the beginning of the breeding season each male tries to occupy a territory and defends it against intruders, particularly against males of the same species. The females, after having reached a certain stage of sexual ripening, swim around and try to penetrate into these territories. This often leads to the formation of a pair. Such a pair stays together for at least several weeks, taking care of the eggs, larvae, and young fish until the latter are about four weeks old. An important activity, particularly at the beginning of parental care, is the fanning of the eggs, which in the cichlids is rather similar to the corresponding activity in the stickleback. Usually the partners take turns in the uninterrupted fanning of the eggs which are deposited on a stone. The partner on duty is fanning most of the time but now and then interrupts its activity to snap at the substrate (by which movements dead eggs are taken away). When the young hatch, they are picked up from the stone and carried to a recently dug pit where they are fanned until the yolk is absorbed and they are able to swim around. The fanning is interrupted by picking up eggs, transporting eggs, and sometimes, when alarmed, by head shaking combined with flapping of the pelvic fins which attracts the swimming young and makes them concentrate near the parents ("calling young"). During this period of parental care other activities are also observed, e.g. digging the pit, defending the territory, and different feeding activities. Detailed records of the occurrence and duration of every activity show that fanning, nipping at the substrate, picking up and transporting eggs, and calling young follow or precede each other more frequently than they follow or precede digging, feeding, or fighting. This suggests that the former activities are in some way or another related to each other and this impression is supported by the fact that the tendency to perform these activities, measured by presenting the adequate stimulus situation, fluctuates roughly synchronously for each of them. Usually this tendency is, at any given time, high in one of the fishes and low in its partner, which is then often somewhere else in the territory, threatening neighbors, feeding, or digging a pit. After some time it can be seen clearly that interest in the offspring increases in the latter partner, it returns to the brood, and frequently, often after a light skirmishing, it relieves its partner which then leaves the eggs or young, though often reluctantly. It is obvious that after some time an internal change takes place in the animal which is away from the eggs, and it is "forced back" to the "nest" while at the same time the parental activities used in caring for offspring become available. After a period (3–15 minutes) of guarding the nest the tendency to perform parental activities seems to decrease again and it is also suppressed by the appearance of the relieving partner. Which activity is performed by the guarding partner depends on external and internal factors. The sight of white, mouldy eggs, for instance, releases nipping off, but this activity also increases in frequency about 24 hours after spawning at the time when the eggs are about to hatch (even if the clutch is replaced by a fresh one). Whether the relieved fish will start digging, feeding or fighting seems again to depend on internal factors, sometimes strengthened by external stimuli. A fish will not show the elements of these three activities intermingled. In the same way, we can distinguish several other groups of related activities by determining statistically which activities follow or precede each other and which activities fluctuate in frequency synchronously and in the same direction. A group which never overlaps with parental activities contains "leading," jerking and quivering movements of head and body, "skimming" (a horizontal, fanning-like movement over a substrate), nipping off the substrate, and spawning, all of which are sexual activities. There are two different kinds of fighting behavior. Intraterritorial fighting occurs usually at the beginning of territory establishment or when an intruding male has penetrated into a territory and tries to maintain itself there. This fighting begins with lateral display in which the fishes, in breeding colors, hold themselves parallel to each other, with the median fins and eventually the branchiostegal membranes erected. In this position they beat towards each other with their tails. Frequently one of the fishes tries to ram the other. At the height of the battle the fishes grip each others' jaws and, by means of vigorous tail beats, pull at each other. The fight ends when one of the fishes gives up. It then immediately loses its breeding colors and assumes a typical "attitude of inferiority," which inhibits further attack and gives it an opportunity to leave the territory. In interterritorial fighting, the initial display is directed frontally. The fishes alternately advance and retreat and occasionally mouth fighting develops from this position. After the fight, each fish withdraws to the center of its territory. A shift of the boundary sometimes results from repeated encounters of this type.

The group of intraterritorial fighting activities is released as a whole when an unknown fish of the same species enters the territory and tries to stay there.

Interterritorial fighting is released by the approach of a neighbor which in many cases may be recognized as an individual, but which can also be distinguished by its behavior or by color patterns typical of a territorial male. The group of sexual activities is released by a fish showing distinct female responses, i.e. a fish which neither attacks nor flees at the initial aggressive display of the resident male (see Baerends and Baerends-van Roon, 1950).

From the above, it will be clear that activities forming a group all have at least one causal factor in common. We have seen that the external situation is often such a factor. That internal factors also play a part is proved by the fact that animals in which different groups of activities are activated often react differently to the same external situation. A *Tilapia* male will react to the same intruding fish with fighting when it is chiefly aggressively motivated or with "leading" when it is chiefly sexually motivated. Its own eggs or young are fanned or rescued by a fish with parental motivation but are often swallowed by a fish when searching for food. Finally, in different moods an animal may react differently to the same situation. We have already mentioned observations of cichlid fish, in which the threshold for parental activities was very much lowered, guarding and leading *Daphnia*, their favorite food, as if they were their offspring.

Further information about the common causal factors underlying the collective appearance of a group of activities can be gained from analysis of the causation of parental behavior in the three-spined stickleback (van Iersel, 1953). This behavior includes activities like fanning, boring in the nest entrance, cleaning the eggs, retrieving eggs, pushing holes in the nest, nest pulling, and retrieving young. Of all these activities, fanning occurs most frequently through the whole parental phase, so it can be used as representative of the group.

An external factor which can release the parental phase in the male stickleback (even without preceding courtship or fertilization) is the presence of fertilized eggs in the nest. Figure 3 shows how the time spent in fanning increases from day to day until the eggs are six days old. Then, about one day before the eggs hatch, the frequency of fanning begins to drop and falls very steeply as soon as the eggs have hatched. The slope of the curve is steeper and the peak higher the greater the number of eggs present. In experiments when a stream of water relatively rich in carbon dioxide but poor in oxygen was siphoned through the nest, there was a rise in the frequency of parental activities such as fanning, boring, and nosing. This suggests that normally the increasing metabolism of the developing eggs causes the gradual rise in frequency of the parental activities. That this is not the only factor determining the amount of fanning is shown when all the eggs in a guarded nest are replaced after some days by an equal number of fresh eggs.

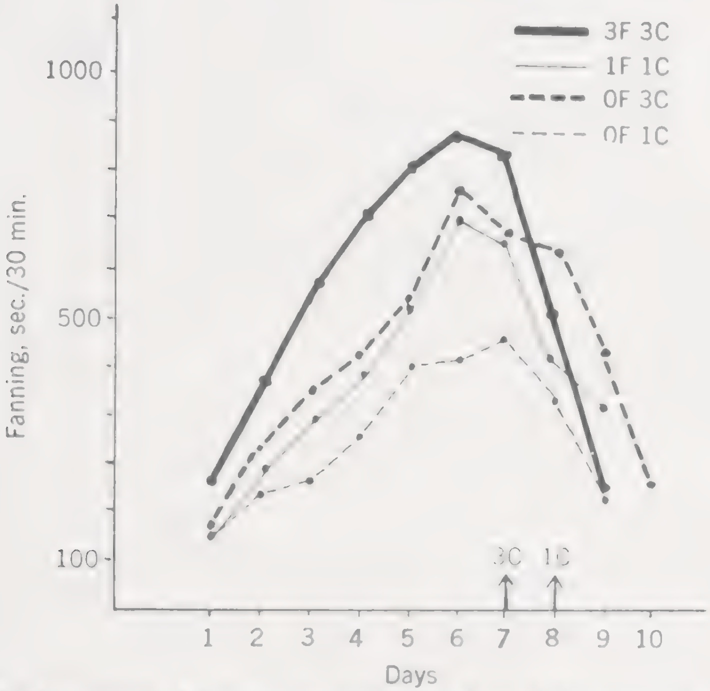

FIG. 3. The frequency of fanning on successive days of the parental cycle, measured as the number of seconds spent fanning during a 30-minute test period. F, indicates the number of times the male has fertilized eggs, C, indicates the number of clutches in the nest. Day 1 is the day after fertilization, the arrow indicates the average hatching day of the young (from van Iersel, 1953).

Figure 4 gives an example of what happens: the fanning curve normally has only one peak, on the day before hatching, but here has two peaks, one on the day before the hatching of the original clutch, the other on the day before the new clutch hatches. This means that besides responding to the existing external situation the behavior of the fish continued to be influenced by stimuli that were removed four days earlier. These earlier stimuli must have brought about a change in some internal factor, which helps to determine the occurrence of parental activities. Apparently this internal factor can vary in strength. Once it has been activated by external stimuli it develops autonomously during the following days. Van Iersel demonstrated experimentally that the later the time when young eggs are substituted for old eggs, the smaller is the influence of the new clutch. This means that the internal factor becomes stronger the longer the fish is in contact with the clutch. The eggs thus not only have

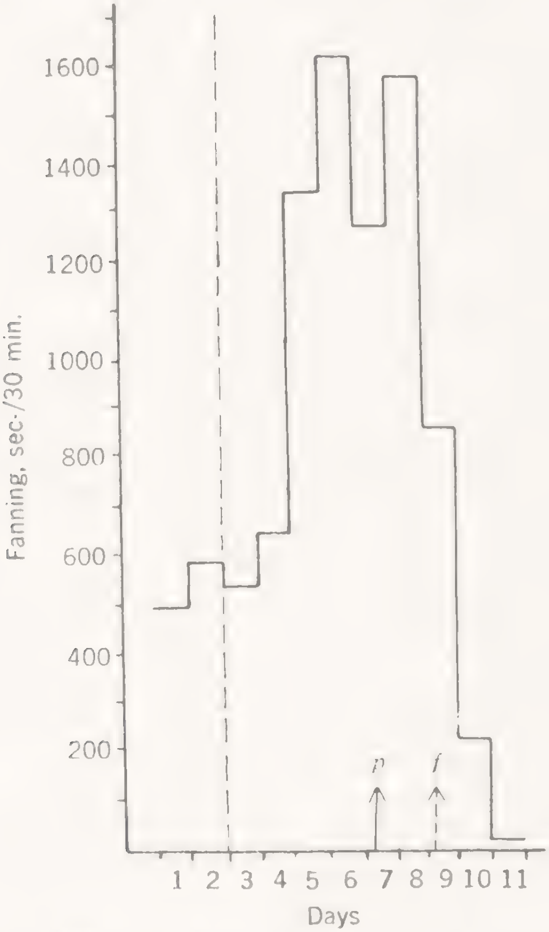

FIG. 4. Frequency of fanning of a male three-spined stickleback which had its original clutch replaced by a fresh one on the second day of the parental cycle. Arrow p = hatching day of original clutch, arrow f = hatching day of foster clutch (from van Iersel, 1953).

a releasing effect on the parental phase but also help to build up the internal factor which, together with the external situation, determines the frequency of occurrence at any moment of the group of parental activities. The words "priming" or "motivating" have been used to describe this building up effect, whereas the term "releasing" is particularly used for the action of starting the behavior pattern. The concept that there are two different effects is closely connected with Lorenz' original idea of how an activity might arise: powered by internal information but normally only released when an external stimulus removes a block. We agree with Hinde (1954) that there are other possible mechanisms which fit the same facts and that "motivating" and "releasing" might very well be the same physiological process (see also Hayes *et al.*, 1953).

Following Tinbergen (1951) and Thorpe (1951) we shall call each mechanism underlying a group of related activities an "instinct." Thus, for the cichlids mentioned above we have to distinguish separate instincts for the group of sexual activities, for each type of fighting, for nest building, for parental care, for feeding, for fleeing, etc. It must be emphasized here that the number and kind of instincts present can be distinguished only after an analysis of the type we have superficially sketched here. Though different instincts often have different ultimate functions, we do not think that the number of functions that can be discriminated should ever be the basis for postulating a corresponding number of different instincts.

The hierarchical organization of behavior was first recognized by Tinbergen (1942, 1950). The grouping of activities has been analyzed only in a few cases but there is ample evidence that a common internal factor always causes this phenomenon. We will call this factor "internal motivation." Usually the animal acts when an adequate external situation appears. The complex of internal and external states and stimuli leading to a given behavior we will call "drive" (Thorpe, 1951).

The term "specific action potentiality" (S.A.P.) has been suggested (Thorpe, 1951; Hinde, 1954) for that state of the animal responsible for its readiness to perform the behavior patterns of one instinct in preference to all other behavior patterns. As we will see later, different instincts may interfere with each other—one instinct, for instance, preventing the activites of another. Hence, the value of S.A.P. for a definite behavior may be quite different from that of the internal motivation of the instinct to which this behavior is subordinate.

While activities can be grouped in units of higher integration, instincts also may be subordinate to instincts of higher order. In the cichlids and the sticklebacks, for instance, nest building, fighting, mating, and caring for offspring only occur after a high level "reproductive instinct" has been activated. In the stickleback this is determined by daylength and temperature (Baggerman, 1957; see also Chap. VII, Vol. I) and, once this internal motivation of higher order has been built up, all the different instincts of lower level can be released by presenting adequate situations. In some cases there are arguments for dividing these sub-instincts again, e.g. in the *Lebistes* male sexual behavior appears to consist of two phases, in the cichlids the parental phase probably includes a separate mechanism for the care of the eggs and one for the care of the young. Besides these instincts of the reproductive super-instinct we find groupings of feeding activities, of cleaning activities, typical fleeing and typical schooling behavior. Unfortunately our knowledge of these is very unsatisfactory as the more spectacular reproductive behavior has received more attention than nonsexual activities.

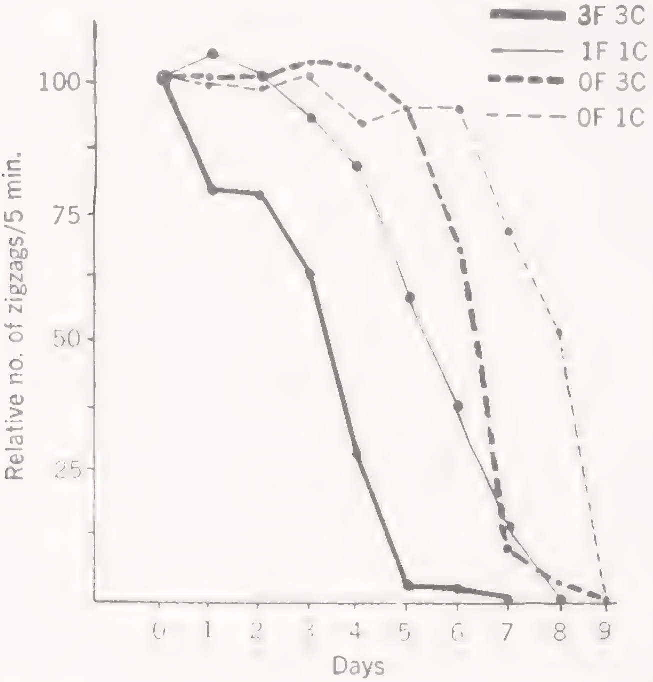

Fig. 5. The frequency of courting on successive days of the parental cycle, measured as number of zigzags per 5 minutes and expressed as per cent of the value on the day of fertilization. F, indicates the number of times the male has fertilized eggs; C, indicates the number of clutches in the nest. Day 1 is the day after fertilization (from van Iersel, 1953).

## 2. Interaction of Instincts

Van Iersel (1953) has analyzed the interaction of two instincts the sexual and the parental, particularly when the activation shifts from one to the other. Estimating the S.A.P. for sexual behavior by counting the number of "zigzags" and the S.A.P. for parental behavior by recording the percentage of time spent in fanning, he showed that the more frequently the male fertilized a clutch, the quicker the S.A.P. for parental activities increased (Fig. 3) and that for courting decreased (Fig. 5). Moreover, the number of zigzags during the transition period falls more quickly when extra clutches are put into the nest by the experimenter (Fig. 5). Therefore, the change from the dominance of one instinct to that of the other is facilitated by performing the consummatory act of the first instinct (the fertilization) and by confrontation with the proper external situation (eggs). These experiments also show that two incompatible instincts inhibit each other.

The interaction of two mutually inhibiting instincts when simultaneously activated has very interesting repercussions on behavior. As long as the internal motivation is relatively low for both instincts, activities belonging to each of them will be performed, but because they are inhibited by the influence of the other instinct their duration or their degree of completeness is low. Such incomplete patterns ("intention movements") of both instincts may be performed successively (as for instance in a hostile encounter at the common boundary of two territories, where the opponents will alternately advance and retreat) or they may be shown simultaneously (as for instance in the behavior intermediate between threatening and leading shown by a *Tilapia* male towards intruders possessing characters of both sexes). However, the more intense the internal motivation of both instincts, the stronger becomes their mutual inhibition and the competition for use of the same motor units. Suddenly, ambivalent behavior is replaced by the performance of an activity that belongs to a third instinct, which so far has not manifested itself. As an example, we will describe what happens when a not quite gravid three-spined stickleback female intrudes into the territory of a male without positively answering the courting of the latter. First the male shows aggressive behavior and sexual behavior alternatively. Then it suddenly swims towards the nest, where it starts fanning (an activity which belongs to the parental instinct) and finally it glues (an activity of the nest-building instinct). Tinbergen (1940, 1952) discovered this phenomenon (simultaneously with but independently of Kortlandt (1940) and called it "displacement" of an activity. Observations suggest that displacement activities occur most frequently when a central nervous mechanism has been stimulated but cannot use its normal outlet because that particular activity is not possible. This situation occurs: (1) in case of a conflict between incompatible instincts (e.g. aggression and flight, possibly combined with sexuality); (2) when the external stimulation necessary for the completion of the activity either does not turn up (e.g. when the female does not follow the leading male) or suddenly ceases (e.g. when at the beginning of a fight, the opponent suddenly flees). These situations, too, might give rise to a conflict.

The physiological mechanism for displacement is still obscure (see e.g., Bastock, Morris, and Moynihan, 1953). For instance in the case of a conflict we do not know whether both instincts or only one of them stimulates the displacement activity. There are indications that each instinct when thwarted activates a definite kind of displacement activity. Thus, for instance, in the example of the stickleback, glueing seems to be the displacement activity of the aggressive instinct, and fanning that of the sexual instinct (van Iersel and Sevenster, personal communication). The same authors have evidence that the original and the displaced activity are slightly different in some quantitative characters. Displacement activities are often incomplete and become more complete the stronger the thwarted motivation (see p. 263). Tinbergen (1940) and Kortlandt (1940) have suggested that a displacement activity is not activated by the instinct to which it normally belongs ("autochthonous activation"), but by at least one of the thwarted instincts ("allochthonous activation"). However, recent unpublished work by Sevenster on the three-spined stickleback indicates that activities satisfying the above criteria of displacement may also be stimulated "autochthonously."

## 3. Appetitive Behavior and Consummatory Act

Let us now consider what determines the kind of activity shown when an instinct has been released or, at a higher level, the kind of sub-instinct activated after stimulation of an instinct of higher order.

One important factor is the external situation. When the male fish is in reproductive motivation, the fighting instinct is immediately activated when a distinctly recognizable male enters the territory. The sexual instinct is activated if a female enters the territory. Which of the different fighting activities or courting activities occurs is also dependent on external stimuli. Even in cases where normally the different activities appear as links in a fixed chain, the sudden presentation of a later link may at once release a more advanced activity. For instance, a sexually motivated male three-spined stickleback which finds a female in the nest without preliminary courting will often respond with "trembling"; this normally occurs only after the male has led the female towards the nest and has indicated the entrance.

Confrontation with the external releasing situation is made much more likely by appetitive behavior. As Craig (1918) first pointed out and as Lorenz (1937a, b), Holzapfel (1940), and Baerends (1941) have later emphasized, the activation of behavior elements (instinctive activities as well as instincts of different order) and the attainment of suitable living conditions (e.g. the right temperature, salinity, or pH, and also the territory or the generally familiar environment) is preceded by searching behavior by which the animal finds the corresponding releasing or satisfying stimulation. Appetitive behavior usually starts as soon as an instinct has been released. Present evidence indicates that in some cases the appetitive behavior is of a general character and may lead to any elements of the next subordinate integration level. For instance, the nipping at eggs by a cichlid parent may, when the egg is loose, lead to bringing it back to the clutch if it is alive and healthy, to devouring it if it is dead or mouldy, and to transporting it to a pit if it has just hatched. In other cases, however, different kinds of appetitive behavior, each corresponding to another subordinate instinct or activity, are performed alternately. Thus a *Tilapia* male which has just established a territory may search for a place to dig a pit, for rivals to fight, or for a female to court. A three-spined stickleback in nest building motivation may show appetitive behavior for digging as well as for collecting material. Hinde (1954) made similar observations on the great tit (*Parus major*). Which of the available appetitive behaviors appears at a given moment seems to be determined chiefly by internal factors, probably by the same factors that cause changes in the threshold of the corresponding activities or instincts.

Thus, in addition to external stimuli, internal factors are also of importance in determining the kind of behavior to be performed. In the sexual behavior of cichlids such as *Cichlasoma*, *Aequidens*, and *Hemichromis*, between early pair formation and spawning, there is a gradual change in the frequencies of appearance of various kinds of courting behavior, even when the external conditions are practically constant. A period in which head jerking dominates is followed by a period with much quivering and nibbling. A little later nipping off reaches a high frequency, while at the same time skimming develops and becomes more and more elaborate until the eggs are deposited and fertilized. This gradual change must be brought about by a shift, probably in intensity, of the internal motivation of the instincts involved. We will illustrate this with two examples from different levels of integration.

In the *Lebistes reticulatus* male different patterns of dispersed melanophores can be distinguished in different phases of the courtship (Baerends *et al.*, 1955). By measuring under standard conditions the frequency of a definite courting activity in males bearing different marking patterns, the occurrence of these patterns could be plotted on a scale representing quantitatively the tendency to perform sexual activities (see Fig. 7, p. 259). From this, the intensity of the internal factor could be deduced from the marking pattern. It thus became evident that during the courtship of the male, definite activities appear at definite internal stages (or marking patterns) while, in addition to this, some of the patterns could be released by special responses from the female.

When the reproductive instinct is activated in the three-spined stickleback, the fish, which winters in sea or in deep fresh water, begins to migrate. This migration, which seems to correspond with the lowest intensity of the reproductive motivation, only stops when the fish arrives in rather warm and shallow water with plenty of vegetation. Then the male establishes a territory and starts building a nest. There are indications that by this time the reproductive motivation has reached a higher intensity stage and a little later, at still higher intensity, full sexual behavior is shown. If, at this stage, the fish is disturbed, for instance by frightening or by lowering the temperature, the behavior "falls back" to the preliminary phases, usually to nest building, but with strong disturbance further back to migration (van Iersel, 1953).

During the performance of a movement, which is a link in a chain leading to some goal (completion of a nest, defeat of a rival, spawning) the specific action potentiality for this behavior often increases and the appearance of the next link is often facilitated. Two factors might increase the internal motivation: (1) feed-back information from proprioceptors stimulated during the performance of the activity; and (2) the effect of the performance on the external situation stimulating further behavior (e.g. the response of a rival or a sex partner). It is extremely difficult to separate these effects experimentally but it can be argued that both factors are involved.

The performance of an activity may increase the internal motivation of the superimposed instinct, but it can also decrease it. If, during prolonged courting, a male *Lebistes* does not elicit a positive sexual response in the female, the courting behavior gradually wanes, passing through the different phases in the reverse order to that in which it usually develops.

When the goal of a behavior sequence is reached, be it the performance of an activity, the release of a subordinate instinct, or stimulation by a suitable environment, the tendency to perform the activities of this sequence suddenly diminishes while, usually at the same time, the tendency to show some other kind of behavior rises. In the three-spined stickleback, van Iersel (1953) measured the S.A.P. for sexual behavior by counting the number of zigzag dances per unit time. The effect, in lowering the S.A.P., of 120 minutes of zigzag dance is about equal to that of 5 fertilizations requiring altogether 30 minutes. Therefore, the ultimate activity of sexual behavior is about four times as powerful in lowering S.A.P. as the introductory courtship. The ultimate activity is the consummatory act and the introductory activity is appetitive behavior in Craig's sense. From the point of view of their effect in lowering the S.A.P. the difference between the two types is only relative (see also Hinde, 1953). The same is true of another distinction which is often given, namely that appetitive behavior is variable and may contain learned and other kinds of intelligent activities, whereas the consummatory act is stereotyped and innately fixed. This is true in many cases but, for instance, in the courtship of the male stickleback, which is a chain of appetitive behaviors leading towards fertilization, the form of each link as well as the order is fixed and stereotyped. Moreover, every link is in itself appetitive behavior with regard to the following link, but a consummatory act with regard to the preceding one.

From the above it will be clear that the principles of coordination and initiation deduced for the lower levels of integration apparently also apply for the levels above that of the instinctive activity, although at the lower levels an activity is caused while at the higher levels a special state results.

### D. Conclusion

To summarize the above considerations we shall now try to give a tentative schematic picture of the general structure of behavior as it appears from observations and experiments like those described above.

All activities are based on motor units which are, as Weiss (1950) has already pointed out, coordinated by centers that control the movements of single muscles. Different muscles are coordinated by centers that each control a special movement of a part of the body. Again, higher coordinating mechanisms control the movements of different parts of the body in relation to each other. In this same way, simple and more complicated reflexes, locomotory movements and more complicated activities are built up. The activities are again coordinated in instincts and these instincts may be grouped under instincts of higher order. Thus, behavior consists of a hierarchy of coordinated patterns; the most complicated integrations at the top, the simplest integrations at the bottom. The same movement can be subordinated to different centers of higher order, for instance, sand-blowing with the pectoral fins in *Cichlasoma nigrofasciatum* is used in feeding as well as in digging a pit for collecting the young. In several species, the same types of fighting may be used in disputes over food, over territories, and over rank order. Locomotory movements, fin positions, and taxes may be used in common by many different centers. The coordinating mechanism, which is presumably located in the central nervous system, may be more or less complicated and is then, respectively, less or more influenced by peripheral stimulation. The stimuli activating the coordinated patterns may be of internal or of external origin. Internal central stimulation may come from superimposed centers, from proprioceptors, and perhaps also from cell groups which produce impulses automatically. It is likely that hormones can act on central mechanisms directly as well as indirectly, e.g. by effecting changes in some organ which are then perceived by interoceptors. All stimuli may evoke activities as well as altering the physiological state of a central mechanism. The taxis forms one part of a coordinated pattern and serves to adapt the other part to the environment. Taxes are chiefly initiated and coordinated by peripheral stimuli, though internal factors play some role. The fixed patterns and instincts of different levels of integration are mainly coordinated by internal factors. Their occurrence depends on external and internal factors in which the latter (which may be intrinsic to the central nervous system) play a very important role. It seems impossible to distinguish sharply between different levels of integration, although the occurrence of introductory behavior may make it possible to discriminate between higher and lower levels.

The ontogeny of behavior patterns has not been studied in fish. The fact that there is relatively little individual variability in the behavior of different members of the same species suggests that there is not much opportunity to acquire motor habits through individually different learning processes. On the other hand we agree with Lehrman (1953) that environmental factors must always influence the development of behavior, just as we know from experimental embryology that they influence the development of organs. Activities, like organs, are the result of genetically controlled interplay between the developing structures and the environment.

Superimposed centers stimulate subordinate centers but also receive feed-back information originating from the activities they have promoted. Centers of the same level may facilitate or inhibit each other. Von Holst's concept of relative coordination gives an insight into how these mutual influences could be brought about.

Some activities serve as appetitive behavior to release other activities. When an instinct is activated there appears the kind of appetitive behavior which has a threshold corresponding to the internal motivation of the controlling center. This may then lead to a situation evoking another kind of appetitive behavior which may be either of the same level (e.g. during courtship of the stickleback) or of a lower level (e.g. migration followed by appetitive behavior for nest building or for courting). It may also lead to a definite activity or to the animal coming to rest.

## III. RELEASING MECHANISMS

### A. Sign Stimuli

As we have seen above, external stimuli are usually necessary to activate movements and instincts and to direct taxes. In a number of cases, those characters of the external situation which are essential for releasing or directing the reaction have been analyzed. In a great many of these cases it turned out that, of all the many physical stimuli sent out by the situation and received by the animal, a limited number are sufficient (sign stimuli). For instance, von Frisch (1942) proved that the fright response shown by minnows when one of them is caught by a predator is released by a single stimulus which is a definite chemical substance released from the skin of the damaged fish.

The situation releasing attack in a hunting pike is slightly more complicated, namely, an object within certain size limits and moving not too slowly.

The external stimuli presented by an animal and releasing a response in a fellow member of the same species are more specific. In now almost classical experiments with dummies, ter Pelkwijk and Tinbergen (1937, see also Tinbergen, 1939, 1948, 1951) studied the properties of the external situations releasing attack and courting in the three-spined stickleback. It was shown that to evoke attack in a male or courting in a female, a model must have a red patch on the underside, a greenish blue upper side and a blue eye. The red belly was by far the most important character. Shape hardly seemed to play a role. To elicit courting in the male, the female models had to be silvery colored and had to have a thickening underneath, corresponding with a swollen abdomen.

Some young cichlids follow the parents for some weeks. Noble and Curtis (1939) and Baerends and Baerends-van Roon (1950) showed that these young are not guided by characters of form but by the typical movement and, in some species (e.g. *Hemichromis bimaculatus*), also by the typical color of the parents.

As internal factors also affect the reaction, the response towards a model is not always uniform. Thus, for instance, Sevenster (1949), when presenting female models to males of the ten-spined stickleback (*Pygosteus pungitius*), obtained a ratio of attack to leading of 1 to 2 with a slender-shaped model and a ratio of 1 to 1 using a model with swollen abdomen. Therefore conclusions can only be drawn from elaborate statistical series of experiments in which precautions are taken to eliminate the influence of fluctuations in S.A.P. Differences in value between models are usually relative.

### B. The Evaluation of Stimuli

The evidence so far available permits us to state that of all the external stimuli received by the animal some have a higher and others a lower value with regard to the release of a definite response. Relatively many have a very low or nondemonstrable releasing value. The quality of a feature, therefore, determines the value or, more exactly, the range within which the releasing value can vary. This latter variation is chiefly caused by quantitative and configurational aspects of the feature. The quantitative aspects are concerned, for instance, with size, intensity, and degree of saturation of the color, or degree of contrast with the background of a morphological structure, or with frequency, duration, or intensity of a movement. An important configurational aspect ("Gestaltmerkmal") is the position of a feature relative to other features.

We have already met an example of the influence of quantitative aspects when discussing fanning in the stickleback. For the release of this activity, carbon dioxide is an important feature. A higher concentration proved to have a greater releasing value than a lower one.

The importance of configurational relations was shown in some experiments on the value of the red underside of the male three-spined stickleback for releasing attack in another male. To a silvery colored model, territorial males responded with attack or with leading in a ratio of 1 to 17, to models with a red underside in a ratio of 6 to 1, and to a model with a red back instead of a red belly (and, therefore, differing in configuration from the second) in a ratio of 1 to 3.

### C. Heterogeneous Summation

In general one can say that with constant internal conditions a response will be stronger when the external situation is richer in features with releasing value. The strength of the response can be measured as frequency, duration, and number of components involved in the movement. For example, Lissmann (1933) and Hess (1953) showed that the value of a model releasing fighting responses in the Siamese fighting fish (*Betta splendens*) increased in effectiveness as more visual features of the fish were added to it.

Seitz (1940) was the first to stress the fact that the intensity of a response is determined only by the total value of the releasing situation irrespective of which of the releasing features contribute to this total. It is possible to replace a set of stimuli by another set without affecting the response. Thus, for releasing fight in the cichlid fish, *Astatotilapia strigigena* Pfeffer, a tail-beating male in asexual dress is equivalent to a quietly posturing male in breeding dress with the median fins erected. Further, a male without the blue breeding color is equivalent to a male from which the conspicuously colored ventral fins and a patch on the dorsal fin have been removed. Seitz called this phenomenon "Reizsummenphenomen," which term was translated by Tinbergen (1948) as "law of heterogeneous summation." Fabricius (1950) has given evidence that this rule also holds for hydrographical factors releasing spawning in fish.

The principle is undoubtedly true in many animals, and has been tested more thoroughly in birds (Tinbergen and Perdeck, 1950; Baerends, 1955). Unfortunately, quantitative work is not yet elaborate enough to justify any conclusions on the mathematical procedure according to which the values of the features are combined. The term "summation" must, therefore, not be taken literally in the mathematical sense.

### D. Definition

Three groups of facts lead us to conclude that the releasing value of a stimulus is not primarily due to special properties or limitations of the sensory organs but must be attributed to the central nervous system. These are: (1) fishes can learn to discriminate very slight visual details, as has been proven in cichlids (see p. 256); (2) features having a high releasing value for one response may have a low value for others; and (3) releasing stimuli from different sensory organs can be combined to give one resultant value, for instance the bitterling (*Rhodeus amarus*), when searching for a mussel in which to deposit its eggs, recognizes it by visual, olfactory, and tactile (current) stimuli.

Lorenz proposed the term "releasing mechanism" (Auslösendes Mechanismus") for the central nervous mechanism that evaluates and combines the information coming from different sense organs into a resultant stimulus activating a movement or an instinct.

This concept has proved useful, but we must realize that it stands for the entire chain from sense organ to the central efferent mechanism. The term does not necessarily mean that the releasing mechanism (R.M.) is localized and it need not be a single unit. The word does not imply an explanation and must not impede further research.

### E. Supernormal Stimuli

Once we know which stimuli have a high value and which properties of each of these stimuli can further increase the value, we can make models containing many features of higher values than ever occur in nature. We can make a stickleback dummy more silver than is natural and increase the swollen abdomen supernormally and such models really prove to have a very high releasing value. This phenomenon of supernormal stimulus situations has been studied more extensively in birds (e.g. Tinbergen and Perdeck, 1950; Tinbergen, 1953a, b; Baerends, 1955).

### F. The Ontogeny of Releasing Mechanisms

Another problem is how these releasing mechanisms originate, and especially how far characters are known innately and how far the R.M. develops through learning. In the introduction, we explained why ethology has not yet progressed very far in studying learning processes. On the other hand, the work of comparative psychologists on learning is, unfortunately, not primarily based on responses of the animal to the natural environment and is not framed in a detailed picture of the natural behavior of wild, nondomesticated animals so it is of little value for our purpose.

In some cases, we know for certain that the capacity to react to particular stimuli with a special response is innate. Young *Hemichromis bimaculatus* follow orange-red discs, even if they have been reared from the eggs in isolation and have never seen adult fish in breeding colors (Noble and Curtis, 1939; Baerends and Baerends-van Roon, 1950). A male stickleback reared in isolation responds to a model in exactly the same way as an experienced one.

Further, we know that the R.M. can be enriched by learning processes. Baerends and Baerends-van Roon (1950) tested daily the "following" response of young *Cichlasoma meeki* against differently colored discs. They compared batches of young which were guarded by the parents (which are gray with a very conspicuous red "throat") with young which were reared in groups without adult fishes. The latter group never showed a preference for models of particular color or shape, but followed all discs presented to them. The young kept with their parents developed at an age of about two weeks a preference for models in which red occurred.

In his study of "*Astatotilapia strigigena*," Seitz (1940) found remarkable differences between the releasing mechanisms for fighting a rival and for courting a female. Males reared in isolation as well as males kept with others of the same species responded to models of males with fighting behavior. However, males reared in a group only courted real *Astatotilapia* females, and never females of other species or carefully prepared dummies, while, on the contrary, males reared in isolation courted very simple models such as a silver ball, if it was moved in the right way. These experiments indicate that the features of the rival male are known innately but that those of the female are mostly acquired through experience, probably in the period when the young are schooling together. They are then all in the nonreproductive dress, which is very similar to the female breeding dress. The males leave the schools when they are beginning to assume breeding colors.

These experiments on fish, together with numerous similar observations made by Lorenz (1935) on birds, strongly suggest that learned knowledge of social companions and other objects is much more complicated and detailed than inborn knowledge. The latter seems to be restricted merely to a few features that are characteristic for the object.

### G. Releasing and Directing Stimuli

In the examples mentioned above, a clear difference has not always been made between the characters releasing an activity and those directing it. In several cases, e.g. the following response in the cichlids, the stimuli serve both functions. But in the alarm reaction of young of mouthbreeding cichlids, for instance, the releasing and directing stimuli can be studied separately. In *Tilapia mossambica*, alarm is released in the young by the approach of some big object or by turbulent currents in the water. They then direct themselves towards the posturing female and if the female is replaced by a model they swim towards this dummy. They direct themselves to the lower parts of the object and to dark patches. (Fig. 6). Having reached a solid surface they push repeatedly

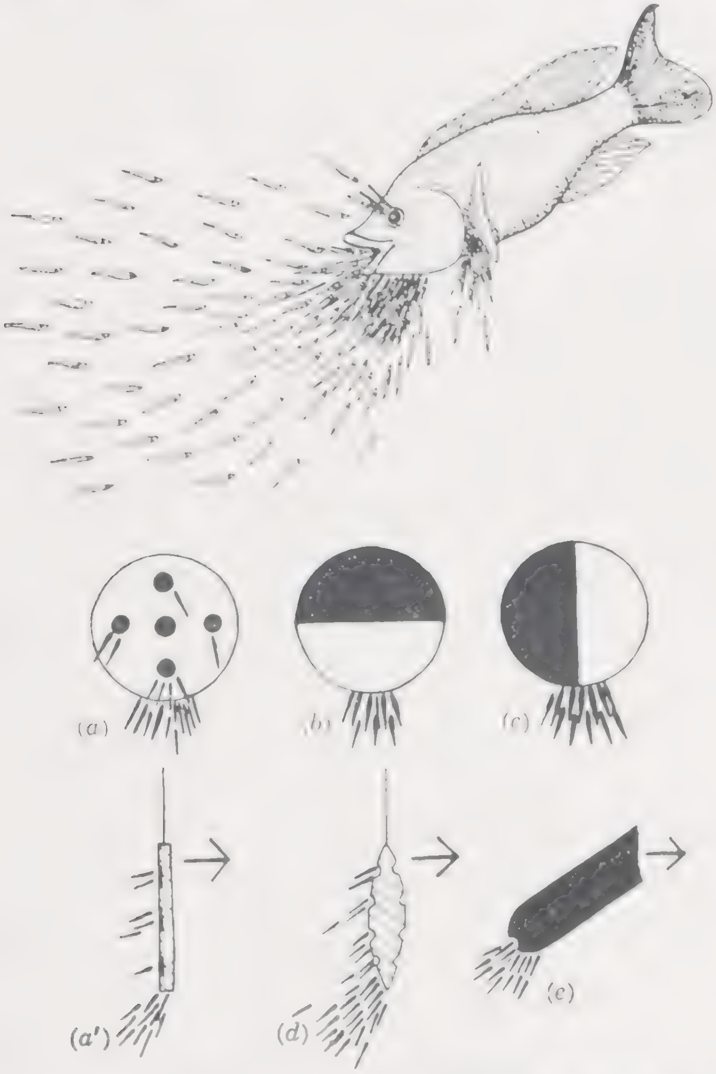

FIG. 6. Some model experiments on the stimulus directing the approach of young towards the female of the mouthbreeding *Tilapia mossambica*. Models a, b, and c are flat discs (cross-section, a′), d is a disc with pits, e is a black-painted test tube with an opening in the bottom. The underside of the object is attractive (a, b, c, d, e) but not because it is the darkest part (b, c). In addition, black spots attract the young (a, a′), while hollows are found by constant pushing against the surface of the model (d, e).

and find holes and penetrate into them. The value of the model increases if it retreats slowly as the real female does. With the real female most of the young, through these simple responses, find the mouth into which they either penetrate themselves or are picked up by the parent. Some young fail to find the mouth and assemble near the black eyes or in the "axils" of the pectoral fins (Baerends and Baerends-van Roon, 1950).

This and other instances from different groups of animals suggest that there is no essential difference between the mechanisms releasing an activity and directing a taxis.

## IV. THE INTERACTION BETWEEN INTERNAL AND EXTERNAL FACTORS

### A. The Causation of "Drive"

We have shown that in general internal and external factors are necessary for activating an instinct or releasing a fixed pattern. We may ask now how differences in the quantities and in the proportions between the quantities of the two kinds of stimulation affect the ensuing behavior. Experiments on the courtship of the male *Lebistes reticulatus* shed some light on this problem (Baerends *et al.,* 1955).

As already mentioned on p. 249 the different marking patterns which develop in the male during courting correspond to different values of S.A.P. for sexual behavior. Consequently these markings can be used as indicators for the strength of the internal factor. The external stimulus needed for courtship is the female; the bigger the female, the stronger is its stimulating effect. In several series of experiments a male bearing a definite marking pattern was confronted with a female of a definite size and the ensuing courting behavior was observed. The interaction of the two kinds of stimulation could thus be studied.

In Fig. 7, the marking patterns are plotted on the abscissa which represents a scale of increasing S.A.P. for sexual behavior. On the ordinate, the size of the female is plotted. Curves have been drawn through points representing different proportions of both kinds of stimuli that produce the same behavior. Two typical fixed patterns were studied this way: (1) following the female, a preliminary courting activity; and (2) sigmoid display, a more advanced courting performance. The lowest and the highest intensity stages of the latter are considered separately. It will be clear from the graph that the same effect can be reached with many different proportions between internal and external stimuli and that the two kinds of stimulation are interchangeable. The curve for a more advanced activity lies above that for a preliminary one (sigmoid compared with following). A high-intensity performance lies above a low-intensity performance (full sigmoid compared with sigmoid intention). Therefore, it is the total amount of information available that, independently of its origin, determines the kind and the intensity stage of the behavior activated. This total information we have called "drive" on p. 245. Here we have to warn the reader that the term "drive" is also used in the sense in which we have used "internal motivation" and "instinct."

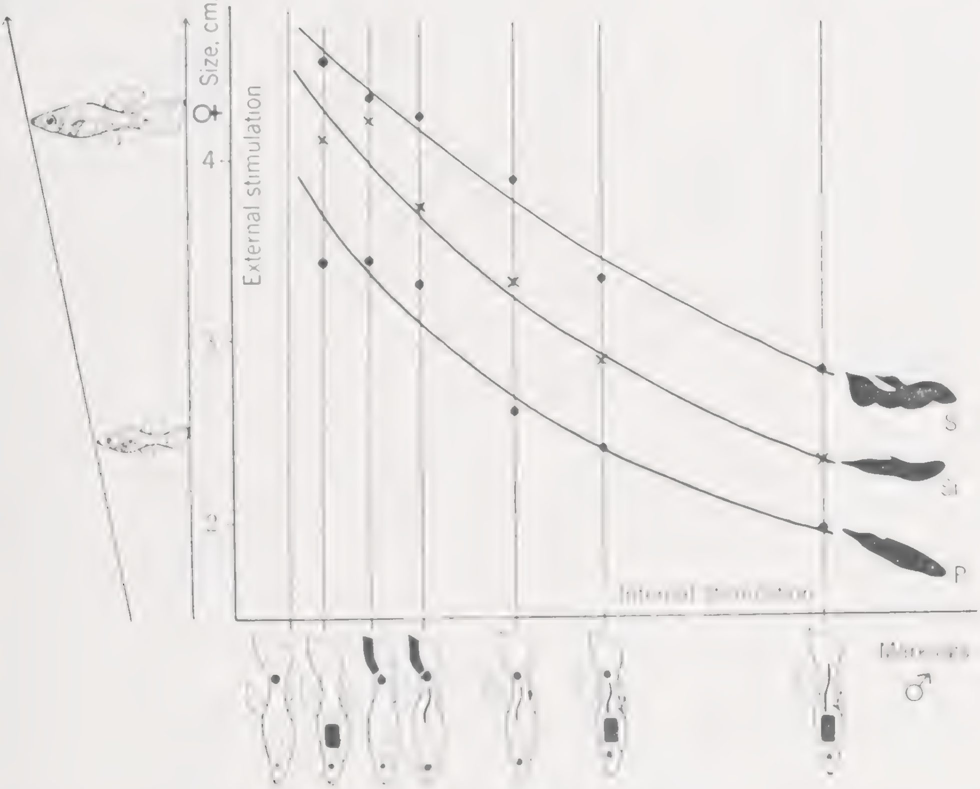

FIG. 7. The combined influence of internal and external stimulation on the kind and completeness of the ensuing courting behavior of the male *Lebistes reticulatus*. The different marking patterns which the male assumes during courtship are used as indicators of the value of S.A.P. for sexual behavior (abscissa); the size of the female is a measure of the external stimulation. P, posturing; Si, sigmoid intention movement; S, fully developed sigmoid (from Baerends, Brouwer, and Waterbolk, 1955).

### B. The Influence of Internal Factors on the Evaluation of a Stimulus

Internal factors can interfere with the effect of external stimuli in different ways. We have already seen that whether an animal will respond or remain indifferent to an external situation depends on the kind of instinct activated. In birds, fishes, butterflies, and spiders, the preference of an animal for each of a series of models varying only quantitatively is influenced by the balance of already activated internal factors. Sevenster (1949) found in the ten-spined stickleback that, in general, the darker a model is the more fighting it will release. All males seldom attack light-colored models but, unexpectedly, some males attack a black model less often than a dark gray one. This is explained by the fact that each model may stimulate flight as well as attack. In a territorial male aggressiveness usually dominates, but if the S.A.P. for fleeing happens to be relatively high compared with that for attack, the black model, representing an intensively displaying male, may stimulate the flight instinct to such an extent that the latter inhibits aggression.

### C. The Influence of Internal Factors on Orienting Reflexes

Von Holst (1950b) found that internal factors can also influence the relative effectiveness of reflexes on an activity. He studied quantitatively how a geotaxis and a phototaxis interact in determining the upright position of a fish in the water.

If the fish is illuminated from the side it takes up a position at an angle to the vertical plane. This angle is the resultant of the geotaxis that tends to keep the fish in the vertical plane and the phototaxis that in this experiment tends to turn the fish into the horizontal plane. Now this angle, and consequently the relative influence of the two taxes, depends on the degree of activation of other instincts in the animal. For instance, when the fish is feeding the phototaxis dominates. At night, when the fish is at rest, the geotaxis is far more important.

### D. Adaptation, Extinction

Finally, we must say a few words about another type of change of responsiveness to external stimuli for which internal factors may be responsible. Prechtl (1953) found that the gaping response of songbirds waned when it was released repeatedly by the same stimulus (for instance, an acoustical one). However, as soon as the stimulus was changed (for instance, for a tactile one) the birds vigorously gaped again. From this it can be deduced that the observed fatigue cannot be localized in the central or peripheral efferent apparatus but must be sought for in the releasing mechanism. Prechtl called this phenomenon "adaptation," in correspondence with the use of this word in physiology. Recently this author (personal communication) has found this phenomenon in the fighting responses of *Betta splendens*. Adaptation is probably of very common occurrence at different levels of integration (see Hinde, 1954; Franzisket, 1953) and it may interfere very much with experiments in which the strength of an external or an internal motivational stimulus is measured. The adaptation is independent of the intensity of the motivation activated in the animal. According to Prechtl, this character should distinguish adaptation from most cases of extinction through learning. It is probably due to such learning processes that a pike, after some unpleasant experiences, stops catching sticklebacks (Tinbergen, personal communication) or that *Lebistes* males, after preliminary attempts to copulate with females of other species, finally restrict their efforts to females of their own species (Haskins and Haskins, 1949, 1950).

## V. RITUALIZATION

We have seen above that the activation of different behaviors is effected through releasing mechanisms that react to definite characters of the external situation. Thus in this way schooling fish react to other fish of their own species, parasitic lampreys find their hosts, a pike reacts to the movement of a prey, and a bitterling (*Rhodeus amarus*) reacts to the optical, chemical, and tactile stimuli of the mussel in which it will deposit its eggs (Tinbergen *et al.,* 1938).

### A. Social Releasers

Often it is of biological significance that an animal should stimulate a definite releasing mechanism in another animal and thus evoke a special kind of behavior. Correspondingly, we find in many cases that animals have developed (by evolution) elaborate characters that fit such situations. Hence, the existence of releasing mechanisms has influenced the phylogenetic development of structural and behavioral patterns able to produce special optical, acoustical, chemical, tactile, and even electrical (Lissmann, 1951) sign stimuli. As it is often also of survival value to animals to react, the releasing mechanisms in their turn have meanwhile evolved and become specialized to respond to these stimuli. Lorenz (1935) introduced the name "Auslöser" for such releasing mechanisms and this term was translated into English as "social releaser" (Tinbergen, 1948; Baerends, 1950). Social releasers are mostly concerned with relationships between members of the same species, e.g. in group behavior, in fighting, in courting, and in the care of young (Baerends, 1952). In group behavior, the releasers help to bring and keep the group together, to provide danger warnings, to promote the finding of food, or to maintain a rank order between the members of the group (Greenberg, 1947). In fighting behavior, they prevent the infliction of serious damage by giving the combats a ceremonial character, by limiting much of the fighting to threatening, and by giving a beaten animal a chance to escape by displaying the signs of inferiority. In sexual behavior, they make possible the selection of a partner of the right species, of the right sex, and in the right physiological condition. Such releasers also promote the synchronization of the sexual cycle of the pair by inhibiting fighting between the partners and probably also by influencing the sexual cycle in the female (Aronson, 1945). Particularly in the cichlids, the young are guided and "called" together by typical releasing movements.

In a few special cases, social releasers are directed at animals of other species (Baerends, 1950). Thus observations by Verwey (1930) and Gohar (1948) on the behavior of the coral fish, *Amphiprion*, which hides in the anemone, *Discosoma*, suggests that the fish, by snapping at the tentacles, causes *Discosoma* to put out of action its cnidoblasts, to which the fish is susceptible.

The angler fishes (Lopheidae) are very remarkable in that they actually "parasitize" on the releasing mechanisms of other species. On p. 253 we mentioned that pike (and the same also holds for other predatory fishes) attack objects moving at a certain speed. The lure of *Lophius* (and probably also the luminescent "angles" of its relatives in the deep sea) correspond to this R.M. Consequently, the lure is attacked by predatory fishes and they are thus led close to the enormous mouth of the angler fish (Wilson, 1937).

### B. The Derivation of Social Releasers

We shall now consider some visual releasers in more detail. The study of social releasers stimulating sense organs other than the eye has hardly begun.

In many cases behavior patterns act as social releasers. Sometimes these are the complete movements which one would expect to be performed under the existing circumstances. Thus, for instance, the slow swimming around of a cichlid parent releases "following" in the young and the fin-digging movement of food-seeking *Cichlasoma nigrofasciatus* makes the young concentrate around it, picking up disturbed food particles. In other cases, only the incomplete intention movement of the expected activity may be performed. For instance, a resident male cichlid which is alarmed by the arrival of an intruder raises its median fins. These movements are similar (Boerema, unpublished data) to the preparation for sudden swimming, where they are necessary for hydrodynamical reasons. Thus, the erection of the median fins in threatening is probably an incipient attack. Particularly in lateral display it gives the optical illusion of an enlargement of the body and as body size is an important factor in winning a fight, we can imagine this threat display to be of survival value to the fish. Other examples are the incomplete spawning movement carried out by substrate-spawning cichlids during courting and the fertilization movement made by the male bitterling when indicating a mussel to the female.

Often the complete or incomplete movements which obviously act as social releasers seem quite out of place under the prevailing circumstances. Fanning is an activity belonging to the parental instinct, but it is used by the sexually motivated male ten-spined stickleback, *Pygosteus pungitius*, (Sevenster, 1949) when showing the nest entrance to the female. Most of the digging done by many cichlids during courtship has no direct function in nest building (which precedes courtship) but functions as a social releaser promoting synchronization of the behavior of the pair. The three-spined stickleback shows the intention movement of digging when threatening a neighboring male, thus marking the boundary of his territory (Tinbergen and van Iersel, 1947). These "irrelevant" activities occur under circumstances when one might expect conflicting instincts to be activated simultaneously in the animal (for instance the aggressive, the flight, and the mating instinct). As mentioned already on p. 247 a conflict between instincts may lead to mutual inhibition of the movements belonging to each of them. As a consequence incomplete activities of both instincts may be performed, sometimes simultaneously, sometimes successively. When, however, the internal motivation of the interacting instinct rises so that they totally inhibit each other, a displacement activity occurs (Tinbergen, 1952). This displacement activity is incomplete at low drive and becomes more complete as the drive rises. Under such circumstances, the intention digging, through which the stickleback threatens, may develop into the digging of real pits. Tinbergen and van Iersel (personal communication) are able to explain the form of the entire courtship ceremony of the three-spined stickleback on these lines. The zigzag dance is successive ambivalence of attack and leading to the nest; showing the nest entrance —when the internal motivation of the sexual instinct is very much increased by the following response of the female, but the internal motivation for fighting is high because of the proximity of the nest—is a displacement activity derived from fanning.

If the same fixed pattern may occur with a direct as well as with an indirect (social releaser) function, then in these two functions the form of the total activity may be different because it may be combined with a different set of taxes, adapting it to the special uses.

Structural patterns also may act as social releasers. Thus, in characid and in cyprinid fish (e.g. in many of the species kept in tropical aquaria) conspicuous patterns are present on the body or on the fins. These fish have pronounced schooling habits; Keenleyside (1955) was able to show that *Pristella riddlei* uses the black patch on the dorsal and anal fin to recognize fellow members of the species. Numerous animals have a R.M. that responds to a circular black spot surrounded by a light ring (an "eye") by activating flight, a reaction which obviously must have survival value in defense against predators. Many fish (like birds and insects) have developed "eye spots" on various places (e.g. opercula, body, tail, median fins). Usually the fish can make this pattern more or less conspicuous, either by changing the state of the chromatophores (*Aequidens maroni*), or by a special movement (raising of the opercula in *Cichlasoma meeki*, fluttering of the tail in *Astronotus ocellatus*). Such enhancement of a structural by a behavioral pattern, making both components become very conspicuous, is found in most cases. Comparison with other species, which lack the special structural patterns, shows that the movements are not new, but that usually they were already present in the group, serving some direct function. The raising of the median fins during threat display is usually associated with pigmentation of the fin in the same color as the body surface. The fluttering movement, which in *Astronotus ocellatus* is enhanced by an eye patch on the tail, occurs in many cichlids during aggressive encounters. During the fanning activity, by which sticklebacks show the entrance of the nest, the ten-spined stickleback displays its white ventral spines, which contrast very much with the black body (Sevenster, 1949). Very striking is the way in which patterns of markings enhance behavior patterns of the courting male of *Lebistes reticulatus*. In the first phase of the courtship the male performs a sigmoid posture and lures the female away from the school. The base of the tail fin is directed towards the female and there is a black spot situated exactly on the base. In the second phase of the courtship, the male takes up a position perpendicular to the longitudinal axis of the female and blocks her further advance. The tail spot has now vanished and a black spot appears laterally on the body in front of the female (Baerends *et al.*, 1955).

The process through which a behavior pattern has acquired in the course of evolution a new function as a social releaser and has changed in relation to this new task is called "ritualization" (Tinbergen, 1940, 1952). Ritualization may thus imply changes in the form of the movement, changes in the taxis components, and addition of new morphological structures. Lorenz (1950) has emphasized that ritualization must also imply a change in the underlying nervous mechanism, making the activity independent of the centers to which it was previously subordinated. An ambivalent activity would, thus, become detached from the original interfering instincts and could make new contacts with superimposed centers or even become independent. Thus, a displacement activity could become incorporated in an instinct that earlier supplied it allochthonously. This could very well be the final stage of the process of ritualization.

Probably the best-analyzed case is the zigzag dance of the three-spined stickleback; this is ambivalent behavior between leading and attack and a new center coordinating the alternation of both components appears to have arisen. From an analysis of the variability in form and length of the attacking and the leading parts, van Iersel (1953, see also Tinbergen, 1951) had to conclude, however, that the zigzag dance is still controlled by the sexual and the aggressive instincts.

It may well be that ritualized intention movements and displacement activities have the greatest effect when they are still supplied by information from their original instincts. For in this case they may work both ways: they function as outlets for the disturbing influences of interfering instincts in the active animal and they suppress the activity of the same instincts in the reacting animal.

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### CHAPTER III: PART 3

# REPRODUCTIVE AND PARENTAL BEHAVIOR

L. R. ARONSON

*Page*
I. Methodology . . . 272
II. Ecological Relationships . 273
A. Meterological Influences . 274
1. Illumination . 274
2. Temperature . 275
3. Barometric Pressure . . . . . . 275
4. Physical Factors and Breeding Cycles . 276
B. Habitat . 276
C. Social Factors . . . 276
1. Breeding Aggregations . 276
2. Territory . . 277
3. The Individual . 278
a. Bonds . 278
b. Sexual discrimination . . . . . . . . . 278
c. Discrimination of species, of mates, of eggs, and of young . . 279
d. Courtship . 279
e. Aggressive behavior . . . . . . . 280
III. Sensory Systems in Sexual and Parental Behavior . 281
A. Visual System . . . . . . . . 281
B. Chemical, Olfactory, and Gustatory Stimuli . 281
C. Auditory and Lateral Line Stimuli . 282
D. Tactile Stimuli . 282
IV. Endocrine Mechanisms . . . . . . . . . . . . . . . 283
A. Anterior Pituitary and Pituitary-like Hormones from Other Sources . 283
B. Gonadal Maturation, Regeneration, and Gonadectomy . 284
C. Treatment with Gonadal Hormones . 286
1. Androgens . 286
a. Adults . 286
b. Immature fish . 287
2. Estrogens . . . . . 287
D. Other Endocrine Relationships . 288
V. Neural Mechanisms . 288
A. Forebrain . 288
B. Hypothalamus . 290
C. Cerebellum . 290
VI. Motor Processes . 290
A. Ovulation . 290
B. Erection . 291
C. Oviposition . 291
D. Sperm Emission . 292
E. Copulation . 292

F. Receptivity . . . . . 293
G. Parturition . . . . . 293
H. Eating Eggs or Young . . . . . 294
I. Yawning . . . . . 294
J. Polygamy . . . . . 294
K. Bisexual Behavior . . . . . 295
References . . . . . 296
Addendum . . . . . 303

## I. METHODOLOGY

The reproductive and parental habits of fishes are manifold, and there are literally thousands of papers describing these behaviors in innumerable species. In some species, courtship behavior is minimal in complexity; the eggs are scattered in an almost random fashion and the sperm are emitted in the general vicinity of the eggs. Others, like the cods and herrings, gather in dense schools with much activity as the eggs and sperm are shed promiscuously into the water. At the opposite extreme, as among the sticklebacks, cichlids and anabantids, courtship, nest building, and prespawning behavior is intricate and prolonged, spawning involves precise synchronizations or bodily contacts, and complex parental procedures follow. In addition, many species exhibit all degrees of viviparity, characterized by courtship and copulation, but parental behavior is usually lacking. A methodical consideration of this multitude of behavioral patterns is beyond the scope of this chapter, but is to be the subject of an extensive monograph now in preparation by Dr. C. M. Breder. Descriptions of breeding behavior can be found in general books on fishes such as Norman (1948) and Kyle (1926) and in journals dealing with subjects in ichthyology and behavior, but for some species the only available accounts are confined to aquarium hobbyist magazines. The present chapter will deal primarily with material considered to be of interest to the physiologist, and such studies are for the most part confined to a small number of better known species.

Most of the reproductive and parental behavior patterns in fishes are limited in range of variability from individual to individual within the species. Such behavior is frequently referred to as "instinctive." As a descriptive designation this term may be useful in distinguishing such behavior from more labile and readily modifiable (so-called learned) activities. However, many investigators maintain that instincts (or a segment thereof) are innate or inborn, purporting to offer information with vague conclusions as to the ontogeny of these patterns. This narrow and outmoded conception of instincts is unlikely to be of value to the physiologist interested in the mechanisms of behavior. For one thing, the terms innate or inborn are defined in a negative fashion by presuming that learning must be absent (Beach, 1955), even though learning cannot always be easily detected or delimited. Moreover, this concept of instinct is not in accord with current developmental theory in that it clings to the futile nature–nurture dichotomy which has little value in embryological studies (Weiss, 1953). But most important, it unavoidably detracts from or conceals fundamental problems in the development of behavior by substituting names for solutions (Schneirla, 1949). Similarly such terms as appetitive behavior, consummatory act, displacement reaction, purpose and drive, while possibly useful as descriptions and for phylogenetic comparisons of behavior in fishes, have frequently been given physiological status as causal explanations of behavior to an extent unwarranted by the available evidence. Physiologists should employ considerable circumspection in the use of these terms and would do well to consult such thought-provoking discussions as Schneirla (1950, 1952), Beach (1951), Hebb (1953), Lehrman (1953), and Kennedy (1954). (See, however, Chap. III, Part 2 for an attitude more favorable to the instinctivist position.)

Students of fish behavior are turning more and more to so-called "tropicals" for experimental studies. Oftentimes the fish are purchased from aquarium shops where the dealer's identification may not always be sufficiently reliable for scientific purposes. Such conditions lead to much confusion. When the identity of the material is uncertain, it is imperative that samples be sent to taxonomic specialists. One should also remember that a number of popular species have been hybridized or highly inbred and selected for characters which are attractive to aquarists but not necessarily of value for behavioral studies.

In studies of sexual and maternal behavior in mammals, quantitative procedures are used extensively and effectively. The adoption of such techniques to deal with the problem of individual variability in the study of fish behavior is relatively recent (e.g. Aronson, 1949). A special recording apparatus has been developed by Clark, Aronson, and Gordon (1954) enabling the observer to record by a touch system, the frequency, order, and duration of any number of rapidly occurring behavioral events. Duncan (1951) discusses the application and reliability of quantitative methods to studies of mating behavior in fishes.

## II. ECOLOGICAL RELATIONSHIPS

Many features of the environment influence reproduction and parental behavior. Some of these effects are relatively direct and, for these, specific physiological mechanisms have been postulated. In other cases the relationships are remote and the underlying mechanisms can at best only be surmised. Nevertheless, their significance to the total behavior of the animal should not be underestimated. Environmental factors may be considered under three major headings: meteorological influences, habitat, and social factors.

## A. Meteorological Influences

### 1. Illumination

The amount and intensity of general illumination plays an important role in the maturation of gonads and the initiation of sexual behavior (see also Vol. 1, Chap. VII). Thus the brook trout, *Salvelinus fontinalis*, normally spawns between October and December when days are shortening. By first artificially increasing and later decreasing day-length, Hoover and Hubbard (1937) induced sexual behavior and ripe spawn in August. The Japanese Medaka, *Oryzias latipes*, lays its eggs daily, just before dawn, but when the periods of light and darkness were inverted, spawning was shifted to the artificial dawn (Robinson and Rugh, 1943; Egami, 1954). According to McNamara (1937), the pike, *Esox*, only spawn on bright days, and this view is supported by Fabricius (1950) who also notes that the assembly of *Esox lucius* at the spawning grounds is stimulated by increased day-lengths and increased light intensities in the spring. When *Gasterosteus aculeatus* that were in winter condition were subjected to continuous illumination, ovulation and spawning were induced in 18 days (Kazansky, 1952). Tinbergen (quoted by Rowan, 1938) also induced premature spawning in sticklebacks by artificial lengthening of the day, and similar results were obtained by Vanden-Eeckhoudt (1947). Aronson (1951) observed that the spawning frequency of the West African mouthbreeder, *Tilapia macrocephala*, maintained at constant temperature in a laboratory greenhouse, dropped noticeably during extended periods of dull weather. Moreover, a major peak of spawning occurred during March and a lesser peak in October, roughly corresponding to the vernal and autumnal equinoxes, which represent periods of greatest daily changes in light. Recent observations of these species under natural conditions in Africa revealed a marked drop in spawning frequency during the periods of heavy rains, coupled in some areas with a retrogression of the gonads. It is significant that during this season a number of factors contribute to a considerable reduction in the intensity of illumination.

Another type of reaction to light is illustrated by the sunfishes (Centrarchidae). These fishes are sexually active primarily on bright sunny days and even the temporary passage of a cloud usually stops such behavior, as the fish retreat to deep water (Breder, 1936).

It is generally supposed that light activates sexual behavior by the well known eye-brain-hypothalamus-anterior pituitary-gonad-gonadal hormone pathway. The following additional possibilities should be kept in mind: (1) radiant energy impinging directly on the brain; (2) sensitivity of the pineal organ to light, as the relation of this organ to phototactic responses has already been established (Young, 1935; Breder and Rasquin, 1950); (3) a dermal sensitivity also related to phototaxis (Thines, 1953); (4) role of illumination as essential for visual direction of sexual and parental responses.

Rasquin and Rosenbloom (1954) discovered that the minimum duration of exposure to daylight necessary to maintain a normal endocrine balance may be extremely small. Robinson *et al.* (1943) believe that the stimulative effect of light causes an increase in general activity which in turn activates breeding reactions.

### 2. Temperature

Although several investigators (Craig-Bennett, 1931; Burger, 1939; Mathews, 1939; Hora *et al.*, 1945, Medlen, 1951) have minimized the importance of light in reproduction and emphasize temperature changes, Bullough (1939) and Merriman and Schedl (1941) point to the joint action of light and temperature. Species differences are obviously important as indicated by the studies of Fabricius (1950).

A reduction in temperature often stimulates spawning activity, as for example in the lake trout *Salvelinus namaycush* (Royce, 1951). Moreover, it is well known among aquarists that many species of characins and cyprinids can be induced to breed in captivity by rapidly lowering the temperature of the water. For other tropicals there seem to be definite minimal temperatures below which the fish will not breed.

### 3. Barometric Pressure

This physical condition does not seem to have any direct influence on breeding in fishes. However, low pressure areas bring rain, which strongly influences the induction of breeding behavior in cyprinids by physico-chemical alterations of the water, especially lowering the temperature, raising the pH, and increasing oxygen tension (Hora *et al.*, 1945). Mookerjee (1945) induced spawning in *Barbus* and *Amblypharyngodon* by oxygenating the aquarium water and Tinbergen (1951) altered parental behavior of sticklebacks by similar treatment. Rain frequently increases turbidity which in turn inhibits breeding in some fishes.

### 4. Physical Factors and Breeding Cycles

It is often stated that the stimuli from fluctuating climatological conditions impinge upon and modify innate breeding cycles. The existence of such cycles is well established, but not as innate patterns. The explanation of these cycles requires investigation of the impact of regularly fluctuating meteorological factors during ontogeny.

## B. Habitat

It is well known that many species of fishes breed in rather restricted areas. During spawning migrations the fish may move from sea water to fresh water, from deep water to shallow zones, from swiftly running water to quiet pools, or from clear bottoms to those of dense vegetation. It is frequently suggested that general features of these spawning grounds such as type of vegetation, quality of the gravel, and degree of water movement act as stimuli for breeding behavior (Fabricius, 1950; Winn, 1953). Thus a male stickleback seems to require the sight of aquatic vegetation for the initiation of nest building (ter Pelkwijk and Tinbergen, 1937). The location of the nest site, and the type of nest that is built is determined in some species by structural features of the bottom (Breder, 1936, 1939, 1940; Winn, 1955), while the presence of a female at a nest site of given characteristics or even the nest alone will stimulate sexual activities in the male (Tinbergen, 1951; Breder, quoted by Beach, 1951; Carranza and Winn, 1954). On the other hand, unfavorable conditions such as turbulent and silt-laden water can temporarily slow down or stop the mating process in the top minnow, *Fundulus* (Winn, 1955). The bitterling *Rhodeus amarus* normally deposits its eggs in the incurrent siphon of a freshwater mussel. If no mussels are present oviposition is inhibited (Bresse, 1950).

## C. Social Factors

### 1. Breeding Aggregations

Some fishes, as for example, herring, mackerel, and many species of cyprinids remain in schools during the breeding period. In these aggregations it is often likely that the stimulative effect of courting and mating (trophallaxis; Schneirla, 1946) spreads throughout the school. In the Atlantic salmon *Salmo salar* (Belding, 1934), the bream, *Abramis brama*, (Fabricius, 1951), and the whitefish, *Coregonus*, immense numbers of individuals crowd together at the breeding grounds. Territories are established, and there is much fighting and thrashing about. Simultaneous spawnings add to the "confusion and turmoil," all of which seems to have high stimulative value. Svardson (1949) states that the spawning of some individuals in an aggregation of *Leuciscus erythrophthalmus* stimulated others, especially the males, as there was always splashing and increased rapidity of swimming of all surrounding fish when one pair was spawning.

## 2. Territory

Defined simply by Noble (1939a) as "any defended area," territory is a factor in the spawning of a great many fishes. The behavior associated with the formation and maintenance of territory has been described for numerous species (reviewed by Noble, 1938). In general, stronger territorial behavior is associated with more pronounced sexual dimorphism and fewer eggs in the spawn. The size of a territory is characteristic of the species, but is subject to variation depending on the body size of the individuals and the density of the spawning population. Since territories are determined primarily on a visual basis, screens such as dense vegetation reduce their size, and a larger number of individual territories can then coexist in a limited area (Fabricius, 1951; Morris, 1952). The jewel fish, *Hemichromis bimaculatus*, identifies its territory and particularly the nesting site by details of size, form, and pattern of objects in the area (Noble and Curtis, 1939). According to Fabricius and Gustafson (1954), territorial behavior can only be studied in aquaria which are at least twice the minimum size of the natural territory of the species. Under special circumstances territorial behavior may be induced in immature individuals of both sexes of sunfishes, as a balance between equally aggressive individuals (Greenberg, 1947), and territories tend to prevent the formation of rigid social structures since individuals in territories are usually dominant (Collias, 1944). Territories are established more commonly at the onset of the breeding season as a place where sexual bonds are formed (Noble, 1939b) and where spawning will occur (Breder, 1936). The commonly observed pronounced aggressiveness towards individuals of the same species as well as other species stems from the familiarity of a territory-holding fish with the physical features of the locality (Breder, 1936).

There has been much speculation concerning adaptive advantages of territory to a species, such as (1) providing a food reserve for the adults and young; (2) regulating the population; (3) expansion of the range of the species if there is insufficient nesting ground; and (4) providing an undisturbed and protected place for pairing and spawning, and enabling partners to find each other. To these we may add a regulative function stemming from the sexually stimulative aggressive interactions.

The origin of territorial behavior has not been adequately investigated in fishes. At the onset, the territory probably represents an area most suited to the physiological requirements of the individual who rapidly develops a special attachment to the site. As other individuals intrude into the area, aggressive actions become the prominent feature of territorial behavior, and hence the aspect of this behavior most intensively investigated. However, it is reasonable to assume that territory and some aspects of territorial behavior can exist even in the absence of all intruders.

### 3. THE INDIVIDUAL

Whereas the courtship behavior of school breeders may have a considerable stimulative effect of a general nature, among strongly territorial breeders and viviparous species, the stimulative processes of the individual become predominant. When females of *Tilapia macrocephala* are visually isolated in separate aquaria, the frequency of spawning will average about three per year. If an isolated female is permitted to see another *Tilapia* in an adjacent aquarium, the average frequency of spawning will rise to seven or eight spawnings per year. The stimulus fish may be either a male, female, or castrated individual of either sex (Aronson, 1945, 1951). In the sargassum fish, *Histrio histrio,* introduction of the male stimulates female egg production and the continued presence of the male leads to regular oviposition cycles (Mosher, 1954).

*a. Bonds.* Territorial behavior facilitates the formation of behavioral interrelationships, or bonds, between partners. These may be very transitory, and may not even persist for the duration of a spawning. In some species bonds may last for only one spawning, while in others, as in the jewel fish they may persist through several spawnings (Noble, 1938). The blind goby, *Typhlogobius californiensis*, is said to remain paired for life (MacGinitie, 1939). The formation of partnerships calls to attention problems concerning the fishes' ability to discriminate species, sexes, gravid individuals, partners, nests, eggs, and young. Of these, discrimination of sex has attracted most interest.

*b. Sexual discrimination.* Among the species studied most extensively, movement is the most important factor in sexual discrimination. Thus Reighard (1913) observed that the territory-holding male logperch, *Percina caprodes*, attacks all intruders. Entering males quickly flee, while gravid females avoid the attacking male and eventually remain in the nest. Failure to flee is emphasized for cichlids by Baerends and Baerends-van Roon (1950) and by Noble (1934) for the sunfish. In the latter an additional factor, namely, bright colors, attracts females and repels males. Sexual discrimination in the jewel fish, *Hemichromis bimaculatus*, is effected by small differences in visually perceived movement (Noble and Curtis, 1939; Seitz, 1942). Here the movements are emphasized by the bright breeding colors. This is also true in the Siamese fighting fish, *Betta splendens*, the stickleback, *Gasterosteus aculeatus*, and the salmons (Noble, 1938). In the goby, *Bathygobius soporator*, Tavolga (1954, 1956a) describes sexual discrimination by the males as a function of the behavior of females in response to male courtship, and a similar conclusion is reached by Baerends and Baerends-van Roon (1950) for *Tilapia mossambica* and other mouthbreeding cichlids.

Breder and Coates (1935) found in a series of experiments that the sexually active male guppy, *Lebistes reticulatus*, will exhibit sexual responses to a variety of objects, which indicates that sexual discrimination is feeble if present at all. However, Noble (1938) points out that this is true only in new situations. When guppies have been in a tank for some time, sexual discriminations are clearly effected by learning processes which are facilitated by striking differences between males and females in color and in size. Several of the previously quoted authors have also shown how learning enters into these discriminations.

Chemical factors in sexual discrimination have been suggested by Breder (1935) for the catfish, *Ameiurus nebulosus*, by Eggert (1931) for *Blennius pavo*, by Roule (1931) for lampreys, and was demonstrated by MacGinitie (1939) for the blind goby. A resident *Astatotilapia* (*Haplochromis multicolor*) male frequently mouths the urogenital region of an intruder. Seitz (1940) suggests that this action facilitates the process of chemical discrimination. Auditory and tactile factors have also been suggested by Breder in the catfish, and Reighard (1903) demonstrated tactile discrimination in the brook lamprey *Lampetra wilderi*.

*c. Discrimination of species, of mates, of eggs, and of young.* Among several species of poeciliids, a learning process in species discrimination is clearly demonstrated (Haskins and Haskins, 1949). In the jewel fish, species discrimination is effected by color differences. Mates are distinguished when in motion by detailed differences in the color pattern, especially of the head. Eggs are distinguished from those of other species by their appearance, but jewel fish are unable to distinguish the eggs of other jewel fish pairs from their own. After the brood has passed the wriggler stage, parents with previous brooding experience can discriminate their young from those of other species, by color pattern and size (Noble and Curtis, 1939). The fact that fishes can make these subtle discriminations conforms generally with the conclusions of sensory physiologists (Walls, 1942) concerning acuity and related properties of their visual system (see also Chap. II, Part 1).

*d. Courtship.* A survey of the modes of reproduction in many species of fishes reveals a considerable similarity in courtship behavior even among widely separated groups. Actions such as following, chasing, mouthing, nipping, swimming to nest site, swimming backwards, erecting fins, spreading operculum and branchiostegal membrane, quivering of body musculature, curving trunk into an arc or S, swishing the tail, working over substratum with mouth or fins, rubbing body or genital papilla, and color changes, appear over and over again in different combinations and often with different behavioral significance. This array of movements seems to approach the limits of the skeletal and muscular properties of fishes, a point that has been dealt with in part by Baerends and Baerends-van Roon (1950). In some fishes, stimulation resulting from these courtship actions produce color and pattern changes, which in turn yield new stimulus conditions (Tavolga, 1954). Many of these movements along with striking color patterns and other structural specializations are frequently described as very specific stimuli (releasers or sign stimuli; Tinbergen, 1951) which are capable of eliciting a particular behavioral pattern in the partner. Thus the red underside of a breeding male stickleback repels other males (Tinbergen, 1951).

Evidence for the specific nature of these stimuli is derived extensively from ingenious experiments with artifacts or crude models which exaggerate certain structural or behavioral features and eliminate others. For example, recently released fry of *Haplochromis multicolor* and *Tilapia mossambica* will swim toward brightly colored disks with black dots simulating head and eyes of parents (Peters, 1937; Baerends and Baerends-van Roon, 1950). Although many experiments using artifacts have been performed, few are genuinely quantitative, so that there exists a consequent danger of oversimplifying the consistency and specificity of the response. Tavolga (1956b) demonstrates that the courtship response of a male goby to a specific visual stimulus is governed in part by previous experiences. A strong predisposition (set) towards courtship response can be induced by a prolonged courtship experience. In experiments with artifacts, such predispositions should be carefully controlled. Moreover, as pointed out by Schneirla (1952, 1956) and Lehrman (1953) there is an unfortunate trend to interpret the results of model experiments in terms of the experimenters' perception of the model as corresponding to an equivalent pattern in the nervous system. What is lacking is the systematic experimental analysis of the effective stimuli in relation to physiological processes in the fish.

*e. Aggressive behavior.* Mouthing, nipping, butting, chasing, and fighting are commonly classified together as aggressive actions. Depending on the particular circumstances in which the behavior appears, it may have a dispersing effect, or on the other hand it may act as a centripetal, cohesive force. Thus, aggressive actions stem from the formation of territories and social organization which may be the early stages of the reproductive process. Aggressive actions sometimes interrupt sexual activities, but under different conditions the same actions may serve to stimulate and coordinate courtship and spawning (Aronson, 1949). Assumptions are often made as to the discrete and conflicting nature of aggressive and reproductive behavior with implications of specifically different physiological bases, although adequate evidence is lacking on all of these points.

Tavolga (1956b) in analyzing the prespawning behavior of *Bathygobius* observes that a number of visual factors of pigmentation and movement, and nonvisual factors as well, operate together to produce a pattern which stimulates, channels, and reinforces the actions of a responding animal. Depending upon the interactions of the stimulus pattern of an animal just introduced into an aquarium with that of a male resident in the same aquarium, the resulting behavior consolidates into combat, courtship, or territorial defense. Moreover the stimulus pattern of the introduced animal sometimes includes actions such as butting and biting which are frequently classified as aggressive.

## III. SENSORY SYSTEMS IN SEXUAL AND PARENTAL BEHAVIOR

### A. Visual System

In a majority of species vision is a dominant sensory modality. It is not surprising therefore that this sensory modality plays a major role in reproductive behavior. In many species, frequent coordinated breeding can be observed when the male and female are in adjacent aquaria, or are separated by glass partitions (Aronson, 1945). Other examples have been cited in the previous section. Even in visually oriented fish such as the jewel fish, however, a blinded male is able to spawn with the female in a coordinated manner, but somewhat imperfectly. However, under this condition brooding behavior is interrupted (Noble and Curtis, 1939). Similarly a blinded male stickleback was able to build a large atypical nest but did not engage in any sexual activity (Leiner, 1929). Unfortunately there have not been many analytical studies on reproductive behavior in species where vision is not the dominant sensory modality.

### B. Chemical, Olfactory and Gustatory Stimuli

Tavolga (1955a, 1956a) demonstrated that a chemical produced by the ovaries of gravid female *Bathygobius soporator* and excreted into the water, promptly stimulates full courtship behavior in males. Experiments utilizing fish with plugged or cauterized nasal passages show this to be an olfactory response. A similar secretion seems to operate in the shad, *Alosa alosa* (Roule, 1931). Breder (1935) suggests the possibility that olfactory and gustatory stimulation may be involved in courtship of catfishes. Seepage through the bottom may furnish limited olfactory and gustatory cues for the location of nests by the char, *Salvelinus alpinus* (Fabricius and Gustafson, 1954).

Other references to chemical stimulants of reproductive behavior do not indicate the sensory system involved. In the stickleback, slime secreted by the male stimulates the male's own courtship (Leiner, 1930). In *Blennius pavo* secretions from cutaneous anal glands in the male serve to attract the female (Eggert, 1931). Secretions of the male lamprey are thought to attract the female (Roule, 1931), but Reighard (1903) found no evidence for this in *Lampetra*. Belding (1934) reports that in the salmon, secretions of the female are said to attract the male. In *Lebistes*, a substance copulin, secreted by the male, may synchronize the estrous cycles of females (Jaski, 1939, 1947), but this finding is questioned by Breder (quoted by Clark and Aronson, 1951) and Baerends, Brouwer, and Waterbolk (1955). In the stickleback a change in the chemical composition of the "nest water" elicits parental fanning. The effective stimulus is probably the lowering of the oxygen content of the water, due to respiration of the eggs (van Iersel, 1953). Lowered salinity seems to stimulate spawning in the Pacific herring *Clupea pallasii* (Outram, 1951).

### C. Auditory and Lateral Line Stimuli

Although it has been known for many years that some fishes produce sounds, it is only with the recent development of underwater audio equipment that the extent and nature of these sounds have been seriously investigated. An extensive survey of this material has been published recently by Fish (1954). Sound production as sexual stimuli, i.e. mating calls, has been described in the gourami, *Trichopsis vittatus* (Beyer, 1931; Stampehl, 1931), and has been suggested as a possible function of sounds produced by species of the sea horse (Gill, 1905), drum fish (Tower, 1908), toadfish and others. In the weakfish *Cynoscion regalis* the sounds are produced primarily by the males who are equipped with special drumming muscles (Fish, 1954). Recent evidence by Tavolga (1956a) indicates that the grunting sounds (100–200 cycles per second) produced by the courting male goby elicit sexual activity in the female.

Dykgraaf (1933) attributed the continuation of courtship in a blinded *Macropodus* to stimulation received through the lateral line organs. In the sunfish (Noble, 1934) and jewel fish (Noble and Curtis, 1939) courtship involves lateral line stimulation. Presumably in these cases, low frequency, sub-sonic vibrations are postulated.

### D. Tactile Stimuli

Mechanical stimulation effective during reproduction is widespread. As noted in the previous section, such items as rate of water movement and texture of bottom often activate or inhibit spawning, and tactile reception is indicated. During courtship, mouthing, biting, butting, tail slapping and other bodily contacts are constantly observed in many species. In the mating of catfishes, tactile stimulation of the barbels evidently plays a part (Breder, 1935). The male stickleback stimulates the female by poking his dorsal spines into her body (ter Pelkwijk and Tinbergen, 1937). Prior to spawning, cichlids and gobies repeatedly rub their sensitive genital papillae and ventral surfaces over the nest, and in this process, the papilla becomes enlarged and erected (Noble and Curtis, 1939; Aronson, 1949; Tavolga, 1954). In many fishes, effective stimulation is apparently achieved through contact of the genital orifices while spawning. The male Siamese fighting fish, *Betta splendens*, not only achieves genital contact but he also wraps his body around the female. In several species of darters (Etheostomidae) the male mounts the back of the female and clasps her with his pelvic fins.

## IV. ENDOCRINE MECHANISMS

In all vertebrates, endocrine secretions from the gonads and anterior pituitary gland are influential in the mediation of sexual and parental behavior patterns (Beach, 1948, 1951). Among fishes, studies in this area are decidedly limited and for the most part represent secondary or casual observations in experiments designed for other purposes (see also Chaps. VI and VII, Vol. I). We can nevertheless see from these investigations that in fishes the basic hormone-behavior relationship is similar to that of other vertebrates. At the same time they reveal certain interesting differences.

### A. Anterior Pituitary and Pituitary-like Hormones from Other Sources

Noble and Kumpf (1936) implanted frog pituitary glands into spayed jewel fish and observed the first stages of courtship. Among intact and gonadectomized males and females that had previously spawned, some of the elements of parental behavior were induced by treatment with prolactin, anterior pituitary extract, pregnancy urine gonadotropins, and whole sheep, codfish, and catfish pituitary glands (Noble, Kumpf, and Billings, 1936, 1938). However, a variety of other substances including NaOH, NaCl, and phenol also stimulated broodiness in a fair number of individuals, so that these results cannot be readily interpreted.

Pickford (1952, 1954) injected purified fractions of pollack pituitary glands into hypophysectomized male *Fundulus* and observed characteristic "S-shaped spawning spasms" within 15 to 20 minutes. Since the time interval was very brief and since the gonads were totally regressed, Pickford concludes that the pituitary hormone must stimulate the nervous system directly in producing these movements. Similar results were obtained using intact males prior to the breeding season, as well as castrated males. Recently, Wilhelmi, Pickford, and Sawyer (1955) identified the active substance with oxytocin and vasopressin from the neurohypophysis. On the other hand, spasms of this type could not be induced with androgens (methyl testosterone), and the stimulated males "showed no interest in the females." It is the opinion of this reviewer that the specific relation of the induced spasms to reproductive behavior needs additional clarification.

Vivien (1941) hypophysectomized male and female *Gobius paganellus* about 1 month before the normal breeding season. Three females (7%) spawned normally 48 hours later. In 30% of the operated males, sperm emission, combat behavior, and guarding of eggs were observed. Tavolga (1955b) hypophysectomized male *Bathygobius soporator* and observed complete loss of combat behavior, courtship, and territoriality. The concomitant reduction in gonadal function cannot account for this loss since castration in *Bathygobius* produces rather different effects (see p. 285). Burger (1941) using hypophysectomized *Fundulus heteroclitus* with regressed gonads, treated them every 3 to 5 days with fresh male *Fundulus* pituitary implants. Within 5 days after the first implantation "sexual antics" were observed and by the 10th day the fish were "in a frenzy of display."

Numerous investigators have induced ovulation and sexual behavior in many species of fishes prior to the normal breeding period, using homoplastic or heteroplastic pituitary implants as well as a variety of anterior pituitary extracts and pituitary-like hormones from other sources. In a few of these studies, such as von Ihering (1935), Baldwin and Li (1942), and Hasler and Meyer (1942) brief observations of sexual behavior are reported. In these cases it is not possible to distinguish between the possible direct action of the hormone on behavioral processes and the indirect action of the hormone via stimulation of the gonads. Production of sexual behavior in mammals by direct action of the anterior pituitary gland was suggested by Smith (1930). An extensive review on the pituitary physiology of fishes by Pickford and Atz (1957) includes a discussion of the relation of the pituitary gland to sexual behavior.

### B. Gonadal Maturation, Regeneration, and Gonadectomy

Among many piscine species, as the gonads mature, nuptial coloration and other secondary sex characters make their appearance, and very shortly thereafter elements of sexual behavior may be observed. Parallel changes occur among the adults of seasonal breeders at the onset of the breeding season. In addition, as noted by Breder (1934) in the blue acara, *Aequidens latifrons*, when the gonads mature, juvenile aggregating tendencies are replaced rather suddenly by centrifugal influences characterized by fighting and territoriality. This correlation between gonadal maturation and the appearance of morphological and behavioral sex characters might lead one to conclude that gonadal hormones are responsible for the observed changes. The experimental evidence cited below partly supports such an interpretation but much more experimental data are needed.

In most vertebrates, gonadectomy of adults results in a gradual or rapid decline in reproductive behavior. However, in certain carnivores, ungulates, primates and in man, a partial or complete pattern of sexual behavior may persist in some individuals for relatively long intervals of time after the operation (Beach, 1948). In fishes, diametrically opposite results are reported. Thus Bock (1928) and Ikeda (1933) state that when castrated males of the three-spined stickleback, *Gasterosteus aculeatus* are paired with ripe females, there is a nearly complete loss of nest-building behavior. Jones and King (1952) described an adult castrated male *Salmo salar* that "showed no interest in the females," in contrast with surgically sterilized males that exhibited normal spawning activity. On the other hand Noble *et al.* (1936) castrated male jewel fish and observed typical courtship, fertilization, and brooding behavior for as many as 202 days after the operation. They obtained up to 13 successive normal spawnings when these castrates were paired with intact females. Comparable results were obtained with male Siamese fighting fish. However, spayed *Hemichromis* and *Betta* females showed no sexual activity when placed with ripe males, but spayed swordtails (*Xiphophorus helleri*) remained "sexually attractive to males." Noble and Borne (1940) state that spayed swordtails maintained their position in a social hierarchy for from 1 to 3 months and castrated males from 1 to $6\frac{1}{2}$ months. Aronson (1951) placed a male *Tilapia macrocephala* on the opposite side of a transparent glass partition from a female. In 15 replications of this situation nests were found on the male side in 17% of the times that the females spawned. When castrated males or intact females were placed opposite the females, nests were found in an equivalent percentage of spawnings, but when spayed females were tested opposite intact females the percentage of nests built was considerably less. Castrated male *Bathygobius soporator* did not exhibit typical combat responses when a strange male was introduced, but courtship toward females was not reduced. However, these castrated gobies were nondiscriminatory and courted males, gravid and nongravid females in a like manner (Tavolga, 1955b).

When castration is incomplete, the testes often regenerate, and the behavioral effects of castration are then reversed (Tavolga, 1955b).

However, when female *Betta* were incompletely spayed, the gonads occasionally regenerated into testes and these animals displayed typical male behavior (Noble and Kumpf, 1937; Kaiser and Schmidt, 1951). Similarly, when chorionic gonadotropin was injected into virgin female swordtails, testes developed and "male-like" attempts at copulation were observed (Baldwin and Li, 1942). When testes were transplanted into spayed female *Oryzias latipes*, they exhibited elements of male behavior (Okada and Yamashita, 1944). Thus the manner and degree of dependence of reproductive behavior on gonadal secretion appears to differ materially in the various groups of fishes. While certain of the above experiments demonstrate that testicular secretions are unnecessary for the persistence of sexual behavior, these hormones may still be required for the ontogeny of this behavior. In fact the experiments on sex reversal support this view. The development of sexual behavior in fish which were gonadectomized while immature might clarify this question.

## C. Treatment with Gonadal Hormones

Various investigators have induced sexual behavior in fishes by treating them with androgenic and estrogenic substances. These have been mixed with the food, dissolved in the aquarium water or injected intraperitoneally or intramuscularly in oily solution. A majority of these studies have been confined to a few species of cyprinodonts. Generalizations concerning the action of these hormones would therefore be premature, especially in view of the variable results of gonadectomy described above. The site of action of the gonadal hormones has been discussed by Tinbergen (1951) who concludes that testosterone acts on the highest nervous centers of the three-spined stickleback. This conclusion is supported by limited evidence and is also contrary to the findings of endocrinologists that the effects of gonadal hormones are usually widespread and manifold (Beach, 1947).

### 1. ANDROGENS

In addition to testosterone propionate and methyl testosterone, pregneninolone (ethinyl testosterone) has been employed in a number of studies. This substance which is related to progesterone and has mild androgenic, estrogenic and progestational capacities in mammals (Burrows, 1945), is strongly androgenic in fishes.

*a. Adults.* Noble and Borne (1940) implanted pellets of testosterone into spayed and intact female swordtails. These individuals rose in position in the social hierarchy, and elements of masculine sexual behavior were observed. These included "attempts at copulation" although the gonopodium (the modified anal fin normally functional during insemination) was only partly developed. Similar effects of androgens on sex behavior were obtained by Régnier (1938), Eversole (1941), Svärdson (1943), Okada and Yamashita (1944), and Laskowski (1953) on the females of the swordtail and related species. On the other hand Jones and King (1952) injected castrated adult male salmon with testosterone propionate and observed following of the female, but no other elements of spawning behavior were evident. Likewise Laskowski (1954) noted that adult female *Platypoecilus variatus* treated with methyl testosterone exhibited only the preliminary male courtship patterns which he designated as phase I and which he believes are associated primarily with the establishment of social relationships. Precopulatory and copulatory activity (phases II and III) were not seen.

*b. Immature fish.* Male behavior has also been induced in immature male and female platyfish and guppies with androgenic compounds (Eversole, 1941; Cohen, 1946; Tavolga, 1949). Here again Laskowski (1954) observed only the first phase of courtship behavior when young female *P. variatus* were treated with methyl testosterone. Hoar, MacKinnon, and Redlich (1952) noted that chum salmon fry and coho fry treated with methyl testosterone did not develop any new behavior patterns, but quantitative changes in the rate of swimming and in the intensity of schooling were recorded. Similarly Tavolga (1955b) suggests that testicular hormone in gobies may alter the threshold of the visual, chemical, and possibly also the auditory sense organs.

### 2. Estrogens

Normal breeding behavior was induced in spayed *Betta* by injection with ovarian extracts, and the same hormone restored most of this behavior in spayed *Hemichromis* (Noble *et al.*, 1936). Brooding of foster young was produced in sexually and parentally experienced male and female jewel fish with corpus luteum extract and with progesterone but estrogenic ovarian extracts were ineffective (Noble *et al.*, 1938). When two-week-old male platyfish were feminized by treatment with α-estradiol benzoate, they were pursued by normal males who "attempted to mate" with them. Although Cohen (1946) interpreted this observation as an example of the induction of female sexual behavior by an estrogen, it requires further examination since in certain circumstances males pursue and thrust their gonopodia towards other males (see p. 295). Moreover sexual discrimination by poeciliid males depends upon a learning through experience of specific structural features of the female (p. 279). It is possible that Cohen's males were responding to the altered structure of the estrogen-treated males rather than to feminine sex behavior. Thus while certain androgens seem to duplicate the endocrine function of the testes, the effectiveness of estrogens is less clear. Moreover Aronson and Holz-Tucker (1947) have produced evidence that the hormone produced by the ovaries of *Tilapia* is not equivalent to the commonly available mammalian and synthetic estrogens.

### D. Other Endocrine Relationships

Thyroxine and desiccated thyroid were partly effective in inducing parental activities in jewel fish (phenol was even more effective) (Noble *et al.*, 1938). Thyroid hormone also plays a significant role in spawning migration (Hoar, 1951) which may be looked upon as a first step in the reproductive pattern (see Chap. VI, Vol. I).

Extirpation of the epiphysis (pineal gland) in the three-spined stickleback caused no observable changes in reproductive and parental behavior (Schönherr, 1955).

## V. NEURAL MECHANISMS

Although physiological studies of central nervous function in relation to behavior in fishes date back over 100 years and include investigations of all parts of the brain and spinal cord (see review by ten Cate, 1935) it is only in recent years that reproductive and parental activities have been included. Thus far only the forebrain, hypothalamus, and cerebellum have been investigated, and the studies are so limited that this field should still be considered as largely unexplored (see also Chap. I).

### A. Forebrain

According to classical theory, the forebrain of fishes is primarily concerned with olfactory processes (Herrick, 1924a, 1948) or the correlation of olfaction and taste (Papez, 1929). This view still commonly cited was developed from neuroanatomical considerations based essentially on studies of the Chondrichthyes and primitive Osteichthyes in which the olfactory system dominates the forebrain. However, in many of the diverse and specialized teleost families, the cerebral hemispheres become greatly enlarged and differentiated in relation to the forward projection of other sensory modalities of thalamic and especially hypothalamic origin. Meanwhile the olfactory mechanism remains rudimentary (Papez, 1929; Aronson, unpublished data), clearly indicating forebrain function other than olfactory (Meader, 1939).

On the basis of embryological evidence, Rabl-Rückhard (1883) concluded that the cerebral hemispheres of bony fishes are homologous with the basal ganglia in the forebrain of mammals, while the mammalian pallium (cerebral cortex plus white or medullary substance) is represented in fishes only by the extensive non-nervous ependymal layer, the choroid tela. This view although widely held for a time was soon shown to be incorrect by Gage (1893), and is no longer accepted. Despite this fact, experimentalists frequently revive this antiquated concept and use for the cerebrum of fishes the erroneous designation "corpus striatum" or "basal ganglia." Herrick (1922) has shown that primordia of all of the major subdivisions of the mammalian forebrain are found in fishes, but on the basis of present evidence, homologies of specific cortical areas are difficult to make.

Tinbergen (1951) advanced the hypothesis that instincts are organized centrally as "a hierarchical system of nerve centers each of which has integrative functions of the 'collecting and redispatching' type." The neurological evidence from fishes cited below does not lend support to the idea of a specific neural center controlling a pattern of behavior and the concept of hierarchical arrangement of such centers has little support from neurological studies in other animals (Lehrman, 1953; Schneirla, 1956).

The first studies in fishes concerned specifically with the function of the forebrain in sexual and parental behavior are those of Noble (1936, 1937, 1939b, 1939c). He reported for several species of cichlid and poeciliid fishes that lesions in the "corpus striatum" (i.e. cerebral hemispheres) resulted in loss of coordination between male and female, and defects in one or more components of brooding behavior. Larger lesions generally involved more components, but the same effect was produced by different lesions and a given lesion produced different effects in subsequent spawnings. Extensive forebrain lesions also caused a loss in dominance behavior. Noble and Borne (1941) reported that unilateral forebrain ablation in the oviparous *Betta splendens* and *Hemichromis bimaculatus* caused no discernable alteration in sexual behavior, and complete extirpation of the forebrain in the viviparous *Xiphophorus helleri* had no effect on mating.

In *Tilapia macrocephala* Aronson (1948) reported that early courtship patterns were only slightly affected by hemi- or total decerebration. Those patterns more immediately related to spawning were markedly reduced in frequency of occurrence, especially by the more drastic lesions. Kamrin and Aronson (1954) studied the effect of forebrain injury on the mating behavior of the platyfish *Xiphophorus maculatus*. All of the sexual acts except gonopodial swinging declined in frequency after operation, but a few males maintained a considerable level of sexual activity even after extensive forebrain deprivations. Pflugfelder (1954) reported that reproduction in *Lebistes* was not prevented after total extirpation of the forebrain. Following forebrain extirpation in the three-spined stickleback, Schönherr (1955) reports that males develop normal breeding coloration. Nest-building behavior is observed, but there is a definite deficiency in orientation (e.g. poking and rubbing behavior occur in atypical places) and the sequence of events is disrupted to the extent that a proper nest is not constructed. Forebrainless males are less aggressive, and territorial defense is weak. However, the operated males do show all of the individual mating reactions up to and including entrance into the nest. In decerebrate females the position of readiness and the behaviors which stem from this are observed. In both males and females there is a noticeable reduction in courtship responsiveness after forebrain ablation. Operated males perform fanning behavior but again orientation is weak.

From the above studies it is evident that the forebrain does not function as the organizer of sexual and parental behavior patterns; rather, it seems to act as a general energizer or facilitator of centers and mechanisms lower in the brain. In the case of the platyfish and stickleback this seems to be true particularly in relation to visually directed responses. Concerning the failure to coordinate, and the variable defects in behavior and orientation described by Noble and by Schönherr, explanation might be sought in lowered responsiveness to specific external stimuli, especially those of the partner.

### B. Hypothalamus

In one male stickleback with extensive hypothalamic injury, Schönherr (1955) reports a marked reduction in all reproductive and parental activities.

### C. Cerebellum

In contrast to the marked effects of forebrain deprivations, ablation of the distal half of the cerebellum (Noble, 1937) or the entire dorsal median body of the cerebellum (corpus cerebelli) of *Tilapia macrocephala* (Aronson, 1948) did not produce any observable changes in spawning or parental behavior. According to Herrick (1924b) the corpus cerebelli in fishes is concerned chiefly with proprioception and the relation of the somesthetic (somastesthetic) and optic systems. Ten Cate (1935) emphasizes the relation of the body of the cerebellum to locomotor abilities. It is nevertheless surprising that such precise movements as fertilization and picking up eggs in *Tilapia* are seemingly unaffected by cerebellar ablations. Evans (1940) considers the cerebellum to be the main center for orientation in space, particularly in relation to the acousticolateralis systems. It may be that as long as the visual system of *Tilapia* is intact, defects due to loss of cerebellar function are not easily discernible.

## VI. MOTOR PROCESSES

### A. Ovulation

This may be defined as the rupture of the ovarian follicles and passage of the eggs into the peritoneal cavity. In teleosts with sac-like ovaries, the eggs remain loose within the sac. This process has not been extensively studied in fishes. Fisheries investigators seem content to describe the reproductive status of females by the macroscopic appearance of the ovaries and by the ease with which they may be stripped, but without direct reference to the follicles. In a few species where ovulation has been followed, behavioral relationships have been found. Robinson and Rugh (1943) state that in *Oryzias latipes* the eggs are laid immediately after ovulation, but this is contradicted by Egami (1954). In the bitterling ovulation occurs one day before oviposition (Bretschneider and Duyvené de Wit, 1947). In *Tilapia macrocephala* the time of ovulation can be predicted from the pattern of sexual behavior exhibited. When the female starts nest passing, ovulation is completed (Aronson and Holz-Tucker, 1949).

## B. Erection

As males and females of cichlids and gobies become sexually active, the genital papilla becomes elongated, swollen, and erect. It also takes on a reddish hue suggesting the vascular mechanism for erection described histologically by Eggert (1931). In the bitterling, which deposits its eggs in the exhalent siphon of a freshwater mussel, the papilla is much elongated and specialized to form a tubular ovipositor. Bretschneider and Duyvené de Wit (1947) explain erection by the presence of an egg blocking the distal end of the tube, and by a contraction of the bladder which forces urine into the tube under pressure.

Prior to coition in the skate *Raja*, the female continuously rubs the anal region of the male with her tail and body wall. According to Friedman (1935) this produces a reflex erection of the claspers and opening of the clasper lips. Leigh-Sharpe (1921) noted the presence of erectile tissue and erection in those elasmobranchs possessing a minimum of skeletal support. He suggests that erection is brought about by a metabolite of the muscles surrounding the clasper gland.

The skeletal and muscular mechanisms for erection of the gonopodium in poeciliid fishes have been described in detail by Rosen and Gordon (1953).

## C. Oviposition

The ovarian sac in most species is composed of elastic connective tissue with little or no muscle fibers. The sac is continuous caudally with a short oviduct terminating at the external opening which is sometimes situated in the genital papilla. A muscular sphincter is often present near the external orifice. Presumably the forceful expulsion of eggs results from the sudden relaxation of the oviduct sphincter and the contraction of the elastic ovarian sac. Oviposition in many species is accompanied by pronounced spasms, quivering, or characteristic contractions of the trunk muscles. By means of these movements it is probable that external pressure is applied to the ovaries.

### D. Sperm Emission

Males of many species also exhibit spasms or undulations of the trunk muscles when spawning. These probably furnish sufficient internal pressure for an emission of sperm when the sphincter near the external orifice of the sperm duct is relaxed. The muscular wall of the sperm duct may induce a flow through peristaltic action. Tavolga (1954) found that some sperm are emitted prior to spawning as the male rubs his erected genital papilla over the nest.

### E. Copulation

In viviparous forms, sperm are transferred from the genital opening of the male to the oviduct of the female. In the viviparous sharks this involves the insertion of the claspers (modified pelvic fins) into the female's cloaca or cloacal folds. Sperm transfer has been studied most extensively in poeciliid species where the anal fin of the male is modified into a copulatory organ, the gonopodium. In the guppy, platyfish, and swordtail (Clark and Aronson, 1951; Clark *et al.*, 1954), insemination never results from the frequent and commonly observed thrusts of the male during which the tip of the gonopodium momentarily touches the genital area of the female. Actual insemination as determined by a genital smear technique, occurs infrequently and is rarely detected except when special observation conditions are established. It is characterized by a prolonged genital contact which may last several seconds. Insertion of the gonopodium is minimal; only the minute tip enters the genital orifice of the female. However, the distal serrae (hook and claw located on the tip of the gonopodium) form an effective holdfast mechanism. When the tip is amputated, copulation does not occur, and males so deprived do not inseminate females. The gonopodium is swung forward and to either side, but in some individuals the direction of thrusting is biased to the left or right side (Aronson and Clark, 1952). As the gonopodium is moved to one side towards the female, the pelvic fin of that side also moves forward alongside the gonopodium. Clark and Kamrin (1951) show that this is necessary for insemination, and probably acts as a support for the protected gonopodium, as well as an aid for the passage of spermatophores. In several poeciliid species, the series of spines near the end of ray three of the gonopodium probably act as sensory receptors which stimulate the release of spermatophores (Rosen and Gordon, 1953). In *Lebistes* a fleshy protuberance, the gonopodial hood is attached to the anterior margin of ray three. It is highly vascularized, contains a prominent nerve plexus and is thought to be a tactile organ functioning during thrusting and copulation (Clark and Aronson, 1951; Rosen and Gordon, 1953).

A detailed analysis of the anatomy, evolution and functioning of the male genitalia in poeciliid fishes has been presented by Rosen and Gordon (1953). Spermatophores are ejaculated by the male when the gonopodium is in the erected position and the base is directly below the genital orifice. Spermatophores pour into a trough formed by the short posterior gonopodial rays that are arranged around the base of rays 3, 4, and 5. The latter form a partially enclosed capillary-like channel along which the sperm pass to the tip of the gonopodium. During genital contact, ovarian fluids enter the gonopodium and reduce the viscosity of the fluid substance binding the sperm together. This releases the sperm and they swim through the gonopodial tube and enter the oviduct.

### F. Receptivity

In order to effect copulation it is usually necessary for the female to cooperate, at least to the extent of not resisting the male. In mammals the facilitative actions of the female associated with copulation are called receptive or estrous behavior. In the guppy, Clark and Aronson (1951) were able to identify receptive females. Kadow (1954) confirmed this by demonstrating differential responses of virgin and pregnant females to sexually active males. Characteristic drifting and circling movements of the female were described as receptive behavior. A cycle of receptivity was postulated with the greatest degree of responsiveness corresponding to the time of fertilization. These patterns of receptivity are also described by Baerends *et al.* (1955). Indicators of receptive behavior are described in the platyfish and swordtail by Schlosberg, Duncan, and Daitch (1949) and Haskins and Haskins (1949).

### G. Parturition

*Lebistes* fry are almost always born at night or early in the morning. The head-first position of emergence is most common but some of the young emerge tail first, particularly when two or three fry are born in rapid succession. The young are expelled with considerable force (Rosenthal, 1951). According to Stolk (1950) the embryos are retained within the ovary by an ovarian occlusion apparatus located at the ovary-oviduct boundary. At the time of birth, the occlusion apparatus opens and the young pass through the oviduct with the help of a peristaltic action of the oviduct wall. The ventral abdominal muscles are not active during parturition and hence intra-abdominal pressure is not considered to be a factor in the birth process.

### H. Eating Eggs or Young

This habit has been described in a variety of fishes, for example by Krumholz (1948) and Jones and King (1949). Among the cichlids where parental behavior is highly developed, the eggs and embryos are frequently eaten when the parents are disturbed. Most of the mouthbreeders retrieve their young after they are released, and here eating the young is infrequent, but in *Tilapia macrocephala* retrieving does not occur and, if the young do not escape as soon as they are released, they will be eaten. When the male of this species picks up newly laid eggs, he will often swallow a few, but females that pick up spawn are more prone to swallowing eggs. In *Lebistes*, Breder and Coates (1932) found that the frequency of eating the newborn was expressible as a direct coefficient of crowding.

### I. Yawning

Fishes frequently open their mouths widely and in a manner suggesting a relationship to yawning in mammals. Breder (1935, 1936) has shown that continued and extensive yawning is an accompaniment of egg protection in numerous species of fishes. In the catfish this behavior may be related to aeration of eggs. Its significance in other species and at other times is less clear. Baerends and Baerends-van Roon (1950) described yawning as a very typical movement observed in all the cichlids that they kept. They include yawning in a group of "movements apparently serving to make the fish feel more comfortable."

### J. Polygamy

Few fishes are monogamous for life, but many species remain paired for the duration of a spawning. On the other hand, various combinations of polygyny and polyandry have been reported, a few of which are described as follows: In *Fundulus notatus*, Carranza and Winn (1954) describe two males spawning with a female at the same time. Supernumerary males participating with a spawning pair have been observed in rainbow daters, *Etheostoma coeruleum* (Reeves, 1907), and log-perch (Reighard, 1913). In the black-sided darter, the eggs of a female are fertilized by several males, but with only one male at a time (Petravicz, 1938). In the salmons and trout, it is not uncommon for two males to spawn simultaneously with a female. Moreover, the males shift from female to female (Greely, 1932; Belding, 1934). Fabricius and Gustafson (1954) observed one male *Salvelinus alpinus* spawn successively with six females. In these species young mature males often participate in the spawning along with the older "cocks." These young males are ripe and may actually fertilize some of the eggs (Needham and Taft, 1934; Jones and King, 1949). Polygamy has also been described in *Bathygobius* where females may spawn simultaneously or successively with a nest-guarding male (Tavolga, 1954). In fact it seems to be characteristic of many strongly nesting species for females to deposit their eggs in several successive nests which are attended to by males (Noble, 1938). Breder (1922) describes two females and three males of *Pseudopleuronectes* spawning together with a mixing of eggs and milt in evidence.

### K. Bisexual Behavior

In the higher vertebrates it is not unusual for males and females to behave sexually toward other members of their own sex and to perform behavioral acts characteristic of the opposite sex. The same phenomenon has been reported frequently in fishes. Thus Carranza and Winn (1954) observed two female *Fundulus* spawning together. Just one female laid eggs. In *Tilapia macrocephala*, sexual and parental behavior patterns of males and females are qualitatively alike, but differ in many quantitative aspects such as the frequency of occurrence of particular activities at given intervals of time before or after spawning. When female pairs of this species were established arbitrarily, bisexual spawning was observed. In every case, one female spawned and the other behaved as a male (Aronson, 1948). Similar behavior can be created with bitterlings when two females are kept together in a tank with a mussel (Duyvené de Wit, 1955). Bisexual behavior in both sexes has been reported in the whitefish (Fabricius and Lindworth, 1954). Pretraviez (1936) observed a male darter mount and clasp another male. Males courting other males have been described in cichlids (Baerends *et al.*, 1950), in the char (Fabricius and Gustafson, 1954) and among the viviparous poeciliids (Schlosberg *et al.*, 1949; Laskowski, 1954). Morris (1952) studied bisexual behavior in three- and ten-spined sticklebacks. When a number of sexually active males were crowded together in an aquarium they courted each other, and some of the males then acted as females. Morris (1955) notes that female behavior in genetic males is less frequently observed and may actually occur less frequently than male behavior in females.

Bisexual behavior need not be thought of solely as the product of an abnormal fish. As shown above, it can be induced in normal fish by special social situations such as pairing two females together. Constant exposure to such situations may condition the animal so that it continues to show bisexual behavior in a normal social environment. Likewise, hormonal imbalance may encourage bisexual activity especially in borderline social situations. The widespread occurrence of bisexual behavior in fishes supports the hypothesis that most if not all fishes possess the neuromuscular and hormonal mechanisms capable of eliciting the reproductive patterns of the opposite sex (Beach, 1942; Aronson, 1949; Barraud, 1955).

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## ADDENDUM

*Drugs and behavior.* Recently several preliminary studies have appeared on the effects of psychotomimetic and psychotherapeutic drugs on behavior in fishes. Abramson and Evans (1954) found that briefly immersing Siamese fighting fish, *Betta splendens,* in lysergic acid diethylamide (LSD 25) in concentrations as low as 1$\mu$g/ml produces a trance-like stupor which is most pronounced in juveniles and is independent of sex. Treated individuals could be aroused by an attacking male and even counterattacked on occasion. After a brief battle they would relapse again into the stuporous condition. Recovery from higher doses was complete within a week. Strangely enough, one of the aftereffects of this drug, according to Evans (1955) is the assumption and retention by the male of a higher position in the social hierarchy. Evans *et al.* (1956) tested 11 related ergot derivatives at comparable dose levels and found that only two, viz., a monobromo derivative of LSD 25 (BOL-148) and a *d*-lysergic acid ethylamide (LAE 32) were partially effective. Bettas treated with these preparations became quiescent for days. The slightest stimulus caused arousal during which time a "typical rage reaction to other fish" was observed. The LSD 25 effect could be blocked by the beef brain extract of Florey and McLennan (1955). Serotonin in the extract was not the blocking agent, nor could it be positively identified with the synaptic inhibitor which Florey and McLennan found in the brain extract (Abramson *et al.,* 1957). Turner (1956) tested 22 species of fresh water fishes mostly "tropicals" and found that only in *Betta splendens* could the aforementioned LSD 25 response be elicited. In addition, he reports that the action of this drug is potentiated by hyoscine and counteracted by pilocarpine. Turner suggests that LSD 25 interferes with the normal action of the neurons of the visual pathways which in turn affects the vestibular mechanism. In contrast to the above study, Keller and Umbreit (1956) report alterations in swimming behavior after exposure to LSD 25 in other piscine species, including guppies, gold fish, and "cave fish." In *Lebistes,* treatment with indole or tryptamine followed by LSD 25 caused periods of abnormal swimming behavior even months later. During this time courtship behavior was not disturbed. These "permanent" alterations in behavior could be reversed with reserpine. A series of tranquilizing drugs and pharmacologically related substances were tested on Bettas by Walaszek and Abood (1956). On the basis of their behavioral effects the drugs were divided into 4 groups. (1) Reserpine-meprobamate—fish did not attack; refused to fight; swimming and appetites normal. (2) Chlorpromazine—fish sedated but reactive to stimuli. (3) Antihistamine—fish fled with violence when attacked; normal response to female absent. (4) Barbiturates—fish showed marked sedative effect.

Although the above studies forecast the opening of an exciting new field of research in brain chemistry in fishes, your reviewer sees an urgent need for a better understanding of the behavior of the fishes studied, for a more analytical approach to the behavioral alterations observed, for better control of external conditions, and for the use of quantitative techniques for recording behavior, particularly those already available.

*Neural mechanisms.* Hale (1956) reports that with the destruction of increasingly larger areas of the forebrain in the green sunfish *Lepomis cyanellus*, aggressive behavior became increasingly difficult to elicit except under conditions of high visual and tactile stimulation. The behavioral pattern remained normal in all respects thus supporting the hypothesis (p. 290) that the forebrain acts as an energizer of lower mechanisms. In the three-spined stickleback removal of the telencephalon of females did not affect the reproductive pattern. Following extensive invasion of the forebrain in males, all of the behavioral elements could still be elicited but effective integration was poor (Segaar, 1956).

*Endocrine mechanisms.* Castration of the three-spined stickleback in the sexual phase caused rapid cessation of all sexual activities, but aggressive behavior persisted (Baggerman, 1957). When given eggs, these castrates exhibited normal parental behavior at a lower level. Testosterone proprionate administered to intact male sticklebacks in winter condition induced nestbuilding, aggressive behavior, and sexual activities (Van Iersel and Burggraaf, quoted by Baggerman). Underyearling fish treated with Pregnyl (mostly I.C.S.H.) developed sexual behavior prematurely. In the green sunfish, Hale (1956) also found no reduction of aggressive behavior after castration.

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## Chapter IV

# THE SWIMBLADDER

F. R. Harden Jones

Page
I. Introduction . . . . . . . . . . . . . . . . . . . . . 305
II. Hydrostatic Function . . . . . . . . . . . . . . . . . . . . 307
  A. Density Determinations . . . . . . . . . . . . . . 307
  B. Gas Secretion . . . . . . . . . . . . . . . . . . 309
    1. Carbon Dioxide and Oxygen . . . . . . . . . . . . 309
    2. Nitrogen . . . . . . . . . . . . . . . . . . . 311
  C. Gas Resorption . . . . . . . . . . . . . . . . . . 311
  D. The Nervous Control of Gas Secretion and Resorption . . . . . . 312
III. The Respiratory Function of the Swimbladder . . . . . . . . . . 313
  A. The Swimbladder as a Lung . . . . . . . . . . . . . . 313
  B. The Swimbladder as an Oxygen Store . . . . . . . . . . 313
IV. The Sensory Function of the Swimbladder . . . . . . . . . . 313
  A. The Sensory Function of the Bladder Wall . . . . . . . . . 313
  B. The Sensory Function of the Connection with the Ear . . . . . . 314
    1. Pressure Perception by the Weberian Ossicles . . . . . . . 314
    2. The Auditory Function of the Connection with the Ear . . . . 314
V. Sound Production by the Swimbladder . . . . . . . . . . . 315
  A. The Mechanism of Sound Production . . . . . . . . . . . 315
    1. The Swimbladder as a Resonator . . . . . . . . . . . 315
    2. Sound Production by the Expulsion of Gas . . . . . . . . 315
    3. Sounds Produced by Vibration of the Bladder Wall . . . . . 315
  B. Sound Production and Hearing . . . . . . . . . . . . . 316
  C. The Biological Significance of Sound Production . . . . . . . 316
VI. The Swimbladder and the Ecology of the Teleostean Fishes . . . . . 316
  References . . . . . . . . . . . . . . . . . . . . 318

## I. INTRODUCTION

The teleostean swimbladder is a hollow organ lying between the alimentary canal and the kidneys, filled with a mixture of carbon dioxide, oxygen, and nitrogen whose proportions often differ greatly from those found in the air. The swimbladder may function as a hydrostatic organ or play a part in respiration, it may act as a sense organ or serve for sound production. It develops as a diverticulum from the mid-dorsal or lateral walls of the gut and in larval cod the swimbladder wall contains peptic glands (Maksutow, 1926; Kudrajaschoff, 1926).

Embryologically, then, the swimbladder is a specialized part of the alimentary canal and Fänge (1953) has proposed a new terminology to bring the anatomical description of the two into line. The probable relation between the layers in the alimentary canal and those in the swimbladder wall are shown in Table I. All the layers of the alimentary canal appear to be represented, with modification, in the swimbladder. The tunica externa, for instance, is probably comparable to the muscularis externa of the alimentary canal, but the muscles associated with the peristaltic movements of the latter are absent and in the swimbladder this layer is largely composed of white fibrous connective tissue. As in the alimentary canal, the loose jelly-like consistency of the submucosa is of great functional significance and in the swimbladder allows the inner mucosa to move independently of the tunica externa.

TABLE I

Probable Relation between the Layers in the Wall of the Alimentary Canal and Those in the Swimbladder

| Layers in the Wall of the Alimentary Canal | Layers in the Wall of the Swimbladder | |
|---|---|---|
| | Description | Terminology Suggested by Fänge (1953) |
| Serosa | Peritoneum covering the swimbladder ventrally | Serosa |
| Muscularis externa | Dense collagenous connective tissue with smooth and striated muscles | Tunica externa |
| Submucosa | Loose, jelly-like layer of fibro-elastic tissue | Submucosa |
| Muscularis mucosa | Layers of smooth muscle cells | Muscularis muscosa |
| Lamina propria | Collagenic connective tissue surrounding smooth muscles of the muscularis muscosa | Lamina propria |
| Inner epithelium | Inner epithelium | Inner epithelium |

The connection between the swimbladder and the alimentary canal, the pneumatic duct, may be lost or retained in the adult. In the physostomatous teleosts the duct remains and the swimbladder is open to the alimentary canal. In the physoclistous teleosts the proximal part of the duct degenerates and the swimbladder is closed. In the closed swimbladder, two morphologically different regions can be distinguished: an antero-ventral secretory region, the gas gland and associated retia mirabilia, and a postero-dorsal resorbent region, the oval. The latter develops from the distal end of the degenerating pneumatic duct, which may, in some fish, take the form of a posterior resorbent chamber. The swimbladder of the eel *Anguilla anguilla* suggests an intermediate condition between the physostomes and the physoclists. In the eel the open pneumatic duct is thin-walled, vascular, and probably corresponds to the oval. In addition the eel has well-developed gas glands and retia mirabilia, and is capable of gas secretion and resorption like a physoclist.

In physostomes blood is supplied to the swimbladder from a branch of the coeliacomesenteric artery and is returned to the heart through the hepatic portal system. In the eel, however, the blood from the pneumatic duct returns to the heart through a vein which joins the right posterior cardinal vein near the sinus venosus. In physoclists the coeliacomesenteric artery and a vein from the hepatic portal system serve the retia mirabilia, oval, and bladder wall. Intercostal branches from the dorsal aorta supply the oval and the bladder wall and blood can be returned to the heart from these regions through the posterior cardinal veins.

The swimbladder is innervated by the sympathetic system through a branch from the coeliacomesenteric ganglion and by branches from the left and right intestinal vagus nerves. The striated muscles often associated with the swimbladder in sound production, are innervated by spinal nerves.

The relation between the teleostean swimbladder and the lungs of the Polypteridae, Dipnoi, and tetrapods is discussed by Goodrich (1930) who concludes that they have both arisen from a posterior pair of branchial pouches. However, it is possible that the teleostean swimbladder may have arisen independently. The early Actinopterygian evolution probably took place under conditions which would have favored an accessory respiratory organ (Westoll, 1944), and from such a structure the swimbladder of the modern teleosts may have evolved.

## II. HYDROSTATIC FUNCTION

### A. Density Determinations

One of the principal functions of the swimbladder is that of a hydrostatic organ. By decreasing the mass per unit of volume it makes the density of the fish more or less equal to that of its environment. There are three main lines of evidence to support this. Firstly, fish with well-developed swimbladders swim easily in midwater in contrast with those in which it is small or absent. Secondly, density determinations, some of which are in Table II, show that the sinking factor (Lowndes, 1937) is close to 1000 when the swimbladder occupies about 7–10% of the total volume in freshwater species and about 5% in marine species. These are the percentage volumes that it would be expected to occupy if it were functioning as a hydrostatic organ (Taylor, 1921; Jones, 1951). When the swimbladder is small or absent the sinking factor is much higher. Thirdly,

TABLE II
Densities, Sinking Factors, and Percentage Volume of the Swimbladder for Different Fishes

| Species | Density | Sinking Factor $\frac{d\text{ animal}}{d\text{ environment}} \times 1000$ | Percentage Volume of the Swimbladder | Reference |
|---|---|---|---|---|
| Freshwater | | | | |
| *Carassius auratus* | 0.991 | 991 | 8.3 | Plattner (1941) |
| *Rutilus rutilus* | 1.001 | 1001 | 9.0 | Plattner (1941) |
| *Fundulus heteroclitus* | 1.000 | 1003 | 7.0 | Black (1948) |
| *Perca fluviatilis* | 1.004 | 1004 | 7.5 | Jones (1951) |
| *Tinca tinca* | 1.008 | 1008 | 7.7 | Plattner (1941) |
| Marine | | | | |
| *Mugil auratus* | 1.010 | 980 | 5.6 | Plattner (1941) |
| *Zeus faber* | 1.018 | 992 | 4.3 | Plattner (1941) |
| *Conger conger* | 1.027 | 1001 | 4.7 | Plattner (1941) |
| *Fundulus heteroclitus* | 1.023 | 1003 | 5.0 | Black (1948) |
| *Gadus luscus* | 1.030 | 1003 | 4.9 | Plattner (1941) |
| *Crenilabrus melops* | 1.033 | 1006 | 4.9 | Plattner (1941) |
| *Onos mustela* | 1.050 | 1023 | 1.1 | Jones (1951) |
| *Pleuronectes platessa* | 1.063 | 1036 | Absent | Lowndes (1941) |
| *Cottus bubalis* | 1.077 | 1050 | Absent | Lowndes (1941) |
| *Callionymus lyra* | 1.084 | 1055 | Absent | Jones (1951) |

fish compensate for the changes in density caused by the decrease or increase in the swimbladder volume which takes place when they move from one depth to another. Compensation is achieved by the removal or addition of gas. In physostomes excess gas escapes through the pneumatic duct which is provided with a sphincter mechanism (Guyénot, 1909), and air can be swallowed at the surface and pumped into the swimbladder through the duct (Evans and Damant, 1929). But, when the swimbladder is closed, the addition or removal of gas can only take place through the bladder wall and the ability of the fish to control the volume of the swimbladder in this way is correlated with the presence of retia mirabilia and gas glands, through which secretion takes place, and the oval, through which gas is resorbed. Evans and Damant (1929) have shown that some physostomes can secrete gas into the swimbladder but they do so very slowly when compared with physoclists. The literature on gas secretion and resorption is extensive and has been reviewed by Koch (1934), von Ledebur (1937b), Jones and Marshall (1953), and Fänge (1953). While the mechanisms of gas secretion and resorption are not yet fully understood, it is now possible to give some account of the various physiological activities involved.

## B. Gas Secretion

Analyses of the swimbladder gas of physoclists taken from deep water give partial pressures of oxygen, carbon dioxide, and nitrogen many times greater than those which exist in the water and the blood, and these gases must therefore have entered the swimbladder against a diffusion gradient amounting to several atmospheres.

### 1. Carbon Dioxide and Oxygen

Gas secretion can be stimulated experimentally by the removal of gas from the swimbladder, an increase in hydrostatic pressure or the attachment of weights to the fish. If secretion is stimulated by the removal of gas from the swimbladder the replacing gas is at first rich in carbon dioxide (Jacobs, 1932; Akita, 1936, Fange, 1953) but later oxygen forms the larger proportion. Secretion is accompanied by vasodilation of the capillaries in the gas gland (Hall, 1924; Jacobs, 1930; Akita, 1936; Fange, 1953), the relaxation of the muscularis mucosa in the gas gland and its contraction in the oval (Fange, 1953). Copeland (1952a) has shown histochemically that the gas gland epithelium contains glycogen, and during gas secretion the metabolic activity of the gas gland increases, as judged by the decrease in its glycogen content (Fänge, 1953). Glycolysis appears to lead to the production of lactic acid (Strittmatter, Ball, and Cooper, 1952; Fänge, 1953; Ball, Strittmater, and Cooper, 1955). The breakdown of glycogen in the gas gland is probably the source of part of the carbon dioxide entering the swimbladder, and some might also arise from the action of an acid on the bicarbonates in the blood (Hall, 1924; Akita, 1936). The enzyme carbonic anhydrase, which is present in the tissues of the gas gland (Leiner, 1938; Sobotka and Kann, 1941; Black, 1946; van Goor, 1948), appears to play an important part in the process, as intramuscular injections of a specific inhibitor of the enzyme prevents gas secretion (Fange, 1953). The pseudobranch is also rich in carbonic anhydrase (Leiner and Leiner, 1940). Copeland (1952b) found that its removal seriously interfered with gas secretion and has suggested that carbonic anhydrase produced there is transported to the swimbladder by the circulatory system. But these are all matters which require further study and there may prove to be a parallel between the part played by carbonic anhydrase in hydrochloric acid production by the gastric mucosa (Davies, 1951) and its part in gas secretion by the swimbladder.

Carbon dioxide production in the tissues of the gas gland will lower the pH of the blood passing through it (Hall, 1924; Akita, 1936) and promote the local dissociation of oxyhemoglobin. Many fish bloods are particularly sensitive to carbon dioxide (Root, 1931; Powers, 1932; Green and Root, 1933; Willmer, 1934; Akita, 1936; von Ledebur, 1937a. Black and Irving, 1937, 1938; Irving and Grinnell, 1939; Black, 1940; Benditt, Morrison, and Irving, 1941; Irving, Black, and Safford, 1941; Root and Irving, 1941, 1943). The phenomenon is probably something more than the normal Bohr effect, the dissociation curves becoming asymptotic with respect to the variable on the abscissa before saturation is reached. This the Root effect as it has been called, means that relatively low carbon dioxide tensions might lead to the dissociation of oxyhemoglobin in the presence of high oxygen concentrations. As can be seen from Fig. 1

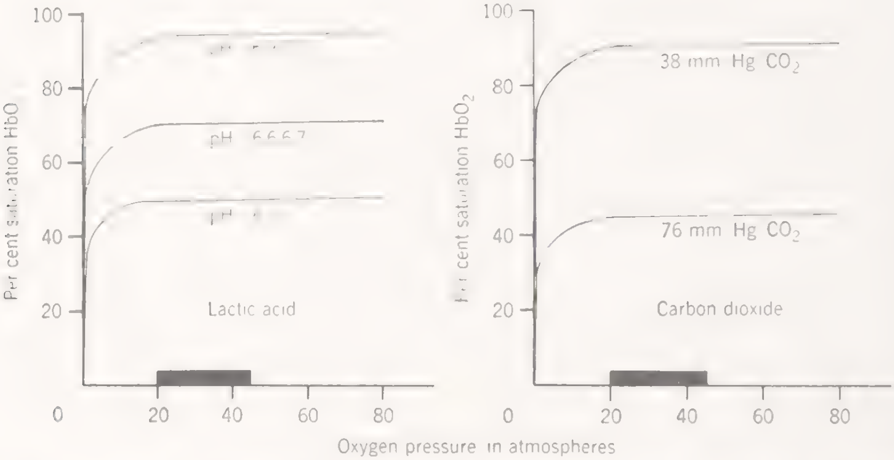

FIG. 1. Oxygen dissociation curves for blood of the Black Grouper, *Epinephelus mystacinus* (from Scholander and van Dam, 1954). The curves showed no change when continued up to 140 atmospheres oxygen pressure. The bar on the abscissa represents the bathymetric range of the fish in atmospheres.

Scholander and van Dam (1954) found evidence of the Root effect in the acidified blood of some deep-sea species even at oxygen pressures of 140 atmospheres. But in other species the blood was fully saturated at pH 6 and lower, in the face of oxygen pressures less than those which would exist in the swimbladder during life. In these cases it seems unlikely that the Root effect can be the fundamental mechanism in oxygen secretion and Scholander (1954) argues in favor of its cellular secretion. The capillary arrangements of the retia mirabilia, acting as a counter-current exchange mechanism (Scholander, 1954), will allow the blood leaving the gas gland to give up its oxygen and carbon dioxide to that coming in (Haldane and Priestley, 1935). The gas tensions in the gas gland could then build up until they exceeded those present in the swimbladder itself. The gases might diffuse through the gas gland whose inner surface is covered by a liquid film (Fange, 1953).

in which bubble formation may take place before the gas is released into the swimbladder lumen.

### 2. NITROGEN

The partial pressures of nitrogen, found in the swimbladders of deep-sea fish suggest that the gas enters against the diffusion gradient. But it is possible that high nitrogen pressures are found because oxygen and carbon dioxide escape from the bladder more rapidly than nitrogen when the fish are being brought to the surface (Copeland, 1952a; Fange, 1953), or that the fish had recently moved from shallow to deep water and were therefore not in hydrostatic equilibrium at the depth at which they were caught (Scholander *et al.*, 1951). However, some of Scholander and van Dam's (1953) analyses cannot be accounted for by the vertical migration hypothesis and the other explanation is not altogether satisfactory. The nitrogen-to-argon ratio in deep-sea fishes approximates that found in sea water and the atmosphere, and this fact is evidence against a chemical secretion of nitrogen (Scholander, 1954).

Powers (1932) has suggested that high nitrogen pressures are found in the swimbladder because nitrogen will diffuse into bubbles of carbon dioxide and oxygen should they form in the gas gland, and that when these bubbles break down, nitrogen will enter the swimbladder regardless of its partial pressure in the bladder gas.

Saunders (1953) has confirmed earlier observations that the swimbladder gas of freshwater physostomes taken from deep water may contain up to 94% nitrogen. The means by which the nitrogen enters the swimbladder and is maintained there is unknown.

## C. Gas Resorption

Gas resorption takes place if the density of the fish is decreased by the attachment of a float, injection of air, or subjecting it to a reduction in pressure. Gas resorption can also be induced by asphyxiation. Gas leaving the swimbladder passes through the oval which opens to allow it to escape, the muscularis mucosa of the gas gland contracting and that of the oval relaxing. Vasodilation also occurs in the blood vessels of the resorbent mucosa (Fänge, 1953). Resorption is slow, physoclists taking several hours to become adjusted to relatively small pressure changes (Brown, 1939; Jones, 1951), and adaptation proceeds most rapidly when the initial concentration of carbon dioxide in the swimbladder is high (Meesters and Nagel, 1934; Brown, 1939). Gas resorption does not present the same problems as gas secretion and can be accounted for by simple diffusion through the oval.

### D. The Nervous Control of Gas Secretion and Resorption

Bilateral section of the vagus nerve running to the swimbladder prevents gas secretion (Bohr, 1894; Kuiper, 1915; Jacobs, 1932; Franz, 1937; Fänge, 1953). Fänge (1953) has shown that electrical stimulation of the vagus nerve leads to the relaxation of the resorbent muscularis mucosa and the opening of the oval. He also found that isolated strips of secretory mucosa contract in the presence of adrenaline but are unaffected by acetylcholine, while the resorbent mucosa contracts in acetylcholine solutions and relaxes in adrenaline and atropine. As atropine inhibits gas secretion (Fänge, 1953), and the gas gland has a high activity of choline esterase (Augustinsson and Fänge, 1951), it is probable that the secretory fibers are cholinergic. Fänge (1953) concludes that both the secretory cholinergic and resorbtive adrenergic fibers reach the swimbladder through the vagus nerve as neither section nor electrical stimulation of the splanchnic nerve appears to affect the muscularis mucosa. Section of the splanchnic nerve leads to a very slight increase in the oxygen content of the bladder gas and thus is probably due to the abolition of vasoconstrictory impulses. The suggestion of the presence of adrenergic fibers in the vagus is not strictly consistent with the idea that it is a parasympathetic nerve, and Fänge (1953) believes that sympathetic fibers controlling gas secretion join the vagus through the intracranial rami communicantes described by Young (1931) in *Uranoscopus*.

As gas secretion and resorption can be induced by the attachment of weights and floats to a fish, which will maintain its position in midwater until adjustment is completed, it is clear that the regulatory mechanisms are not necessarily dependent upon changes in tension of the bladder wall, which, surprisingly, appears to have little or no sensory function. Neither do the Weberian ossicles play any part in the control of gas secretion in the Ostariophysi (Schreiber, 1934). Within limits, fish will maintain their position in midwater by compensatory fin movements (Baglioni, 1908; Meesters and Nagel, 1934; Brown, 1939; Jones, 1952) until adjustment of the swimbladder is completed. Copeland (1952c) suggests that the fin movements might initiate proprioceptive stimuli which would lead to the secretion or resorption of gas. However, Fänge (1953) has shown that secretion still occurs when the pectoral fins are denervated by section of the brachial plexus and it is almost certain that the compensatory swimming movements and the processes of gas secretion and resorption are induced by exteroceptors which are stimulated when the fish, no longer in hydrostatic equilibrium, rises or falls in the water.

## III. THE RESPIRATORY FUNCTION OF THE SWIMBLADDER

### A. The Swimbladder as a Lung

In some physostomes the swimbladder may function as a lung. Such fish live in swamps and pools where the carbon dioxide tension is high and that of oxygen low. An account of the use of the swimbladder as a lung is given in Chap. I, Part 2, Vol. I.

### B. The Swimbladder as an Oxygen Store

Physoclists, and physostomes whose swimbladders are not modified as lungs, may draw upon the oxygen in the swimbladder as an emergency store in times of shortage. This has been demonstrated for a number of species, such as the banded and the yellow perch, *Perca fluviatilis* and *P. flavescens*, the toadfish, *Opsanus tau*, the tench, *Tinca tinca,* and the goldfish, *Carassius auratus*, by Moreau (1876), Pearse and Achtenberg (1918), Hall (1924), Toryu (1927), Khalil (1937), Safford (1940), Plattner (1941), Black (1942), and Fänge (1953). But as Hall (1924) and Khalil (1937) have shown that the oxygen removed from the swimbladder would only meet the oxygen requirements of the fish for a few minutes, the swimbladder would appear to have little functional significance as an oxygen store. However, in deep water the situation may be different as the actual amount of oxygen in the swimbladder will be greater than when the fish is at the surface.

## IV. THE SENSORY FUNCTION OF THE SWIMBLADDER

As compression and rarefaction of the bladder gas occurs when the fish is subjected to pressure changes, the swimbladder could function as a manometer, barometer, or a hydrophone. Changes in the swimbladder volume could stimulate proprioceptors lying in or about the bladder wall and when the swimbladder is linked to the membranous labyrinth there is the possibility that this connection might serve as a pressure receptor.

### A. The Sensory Function of the Bladder Wall

Baglioni (1908) suggested that the swimbladder functioned as a sense organ which enabled a fish to maintain a steady depth when in midwater. If the fish swam above or below the depth at which it was in hydrostatic equilibrium, changes in tension of the bladder wall caused by the expansion or compression of the enclosed gas would lead to compensatory swimming movements which would bring the fish back to its original level. This seems a very reasonable hypothesis and it is most surprising to find that there is no convincing evidence to support it and that most of the experimental evidence is against it (Remotti, 1924, Franz, 1937; Dijkgraaf, 1942; Jones and Marshall, 1953). In physoclists, the compensatory swimming movements, like the processes of gas secretion and resorption, appear to be independent of changes in tension of the bladder wall. But in physostomes, Dijkgraaf (1950) has shown that proprioceptive stimuli from the bladder wall are probably concerned in the release of gas bubbles when the fish are subjected to pressure reductions, while Koshtojanz and Vassilenko (1937) and Vasilenko and Livanov (1936) have obtained results which suggest that the bladder wall may have some sensory function.

## B. The Sensory Function of the Connection with the Ear

As the anatomical and functional relationships between the swimbladder and the labyrinth are discussed fully in Chap. II, Part 2, reference to them here will be brief.

### 1. Pressure Perception by the Weberian Ossicles

In the Ostariophysi the anterior chamber of the swimbladder is linked to the pars inferior of the ear through the Weberian ossicles. Bridge and Haddon (1893) suggested that the mechanism would increase a fish's sensitivity to volume changes of the swimbladder and allow a finer control over the escape of gas through the pneumatic duct. After somewhat inconclusive results had been obtained by Guyénot (1909), Kuiper (1916), Kokas (1932), and Franz (1937), later work by Moehres (1940) and Dijkgraaf (1941, 1942, 1950) has shown Bridge and Haddon's suggestion to be correct. When subjected to a slow reduction of pressure, intact minnows release gas bubbles after a decrease of about 32 mm. Hg; this threshold rises to 39 mm. Hg after breaking the chain of ossicles. However, no experiments have been made to test Sagemehl's (1895) hypothesis that the Weberian ossicles function as a barometer, although some fish, particularly loaches (Philipsen, 1913; Kyle, 1926; Wunder, 1936) are said to be sensitive to changes in atmospheric pressure.

### 2. The Auditory Function of the Connection with the Ear

(See also pp. 176–182.)

It is now well established (for literature see von Frisch, 1936; Jones and Marshall, 1953; Kleerekoper and Chagnon, 1954) that fish in which the swimbladder is connected to the pars inferior of the ear, either by the Weberian ossicles or diverticula of the bladder itself, respond to sounds of higher frequency and lower intensity than those in which such a connection is absent. It will be of interest to see if this is also true of fish in which the connection is to the pars superior of the ear, as electrophysiological work by Pumphrey (1939) and Lowenstein and Roberts (1948) has shown that the utriculus in certain teleosts and in the elasmobranch *Raja clavata* are sensitive to vibrations.

## V. SOUND PRODUCTION BY THE SWIMBLADDER

It is well known that the swimbladder may play an important role in sound production (Dufossé, 1874; Sorensen, 1884, 1895; Tower, 1908; Hagman, 1921; Greene, 1924; Burkenroad, 1931; Hardenberg, 1934; Rauther, 1945) but only recently have physical data on the frequency and intensity of fish noises become available (Loye and Proudfoot, 1946, Dobrin, 1947; Knudsen, Alford, and Emling, 1948; Johnson, 1948; Fish, Kelsey, and Mowbray, 1952; and Fish, 1954).

### A. The Mechanism of Sound Production

#### 1. The Swimbladder as a Resonator

The swimbladder may act as a resonator for sound produced by neighboring organs. For example, in the Balistidae, the trigger fishes, the swimbladder serves as a resonator for sounds produced by the stridulation of the cleithrum against the post-clavicles and the grating of the pharyngeal teeth (Sorensen, 1894; Mobius, 1889; Cunningham, 1910; Fish, 1954); or as in the carangids and haemulids (Burkenroad, 1930), for sounds produced by the pharyngeal teeth alone.

#### 2. Sound Production by the Expulsion of Gas

The release of gas bubbles through the pneumatic duct is often accompanied by sounds, and these have been described for the European eel, *Anguilla anguilla*, and various Ostariophysi by Dufossé (1874), the minnow, *Phoxinus phoxinus*, by Dijkgraaf (1932), and the American eel, *A. rostrata*, by Fish (1954).

#### 3. Sounds Produced by Vibration of the Bladder Wall

In certain catfish, the bladder wall may be vibrated by an elastic spring apparatus (Müller, 1842; Bridge and Haddon, 1893; Sorensen, 1884, 1895); this is a modification of the transverse processes of the fourth vertebrae, which are attached posteriorly to the bladder wall and anteriorly to the occipital region of the skull by a powerful muscle. In other catfish (Bridge and Haddon, 1893; Hardenburg, 1924), the drumming muscles run directly from the skull to the bladder wall (Hagman, 1921). In the sciaenid, *Micropogon undulatus*, the muscles are attached to the body wall (Tower, 1908). In another type of vibratory mechanism the external muscles are attached entirely to the bladder wall. Examples are gurnards belonging to the genus *Trigla* (Dufossé, 1874; Rauther, 1945), and the toadfish, *Opsanus tau* (Tower, 1908). The sound-producing muscles are striated and are innervated by spinal nerves (Rauther, 1945). Other structural modifications of the swimbladder often associated with sound production are transverse diaphragms dividing the bladder into two communicating chambers, or the presence of numerous finger-like diverticula.

### B. Sound Production and Hearing (See also pp. 176–182)

Fish which have no connection between the swimbladder and the ear do not respond to frequencies higher than 2000 to 3000 c.p.s., and when the connection is present, as in the Ostariophysi, the upper limit may lie between 7000 and 10,000 c.p.s. Fish noises range in frequency from 26 to 4800 c.p.s. The frequency spread of sounds produced by stridulation is wide and as a rule the maximum energy is located in the wave bands, between 400 and 800 c.p.s., in contrast to sounds produced by vibratory mechanisms, where the maximum energy mainly lies between 75 and 150 c.p.s. So the frequency of the sounds produced by fish lies within the range over which they can hear.

There is a similar correlation with regard to intensities. With good performers, sound pressures of between 100 to 120 db. above 0.0002 dynes cm$^2$ have been measured within 30 to 60 cm. of the fish (Fish, 1954). These values may be compared with threshold levels of 20 and 50 db. above 0.0002 dynes/cm$^2$ for fish with and without connections between the swimbladder and the ear, respectively.

### C. The Biological Significance of Sound Production

Fish (1954) has analyzed the character and significance of sound produced by a large number of North Atlantic species and has concluded that they serve for communication, are of particular importance during the breeding season, and may be used for offense or defense. A parallel can, perhaps, be drawn with the birds. Sciaenids have dawn and dusk choruses, *Opsanus tau* appears to have a mating call, and the male will give coarse grunts if disturbed when guarding the nest. To these functions of sound production there may be added another, that of orientation. Griffin (1950) has recorded a fish call followed by what appeared to have been its echo from the bottom in deep water, and he has suggested that the fish may have been making use of some system of echo sounding.

## VI. THE SWIMBLADDER AND THE ECOLOGY OF THE TELEOSTEAN FISHES

The principal function of the swimbladder is that of a hydrostatic organ and as might be expected, it is best developed in fish which are active midwater swimmers. The swimbladder is rarely lost in freshwater species, exceptions being some bottom living darters, the Etheostominae, and certain cottids living in deep lakes. But the swimbladder may be reduced in size in bottom living species, and those living in hill streams or swiftly running water, to whom a hydrostatic organ would be of little advantage. In the Ostariophysi, reduction of the swimbladder is achieved by the loss of the posterior chamber, the anterior chamber and the Weberian ossicles being retained, although the latter may be reduced in number.

In the neritic zone of the sea, the swimbladder is often absent among fish living between the tide-marks. The swimbladder is generally present, and closed, in midwater dwellers, while in the pelagic surface dwellers of the coastal waters, such as the clupeoids and salmonids, it is open. The swimbladder is closed in surface dwellers in the oceanic zone of the sea and generally well developed in the bathypelagic forms living down to 500 meters. The swimbladder may be lost or converted into a fat storing organ in deeper living species (Marshall, 1950) and is lost in certain bottom dwellers of the oceanic region.

It may be significant that physostomes predominate in fresh water, while there are few in the neritic zone of the sea and probably none in oceanic waters. With the exception of the eels, which are functionally physoclists, physostomes can only secrete gas very slowly, if at all, and gas lost from the swim bladder through the pneumatic duct can only be replaced at the surface. Physostomes living in deep water might be unable to make the long journey to the surface to make good the loss, and if they did, enough air must be taken in to allow for the compression that would take place on the return to deep water. Closure of the swimbladder and the development of gas glands and retia mirabilia may have brought a new measure of independence from the surface that may have been necessary for the successful colonization of deep water. In this connection it is worth recalling that the bathypelagic stomiatoid fishes have closed swimbladders, although they belong to the physostomatous order Isospondyli. In the Salmonoidea, members of the same order, the swimbladder is similarly closed in the deep water *Argentina* and the Microstomidae. But closing of the swimbladder must lead to a restriction on the rate at which fish can move from one depth to another (Jones, 1951; Scholander *et al.*, 1951) and, although an attempt has been made (Jones, 1952) to get an estimate of the extent of the restriction, the problem is one to which there is not yet a satisfactory answer. In very deep water, so much energy may be required to secrete gas into the swimbladder as to make it uneconomical, and this may account for its loss or conversion to a fat-storing organ in certain bathypelagic forms.

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## CHAPTER V

# ELECTRIC ORGANS

### R. D. KEYNES

Page
I. The Discovery of Electric Fishes . . . . . . . . . . . . . 323
II. The Anatomy of the Electric Organ . . . . . . . . . . . . . 324
III. The Origin and Development of Electric Organs . . . . . . . . 328
IV. The Characteristics of the Electric Discharge . . . . . . . . 329
V. The Discharge of a Single Electroplate . . . . . . . . . . . 332
VI. The Nervous Control of the Electric Organ . . . . . . . . . . 336
VII. The Biological Function of the Electric Organ . . . . . . . . 338
VIII. The Immunity of Electric Fishes to Their Own Discharge . . . . 340
References . . . . . . . . . . . . . . . . . . 340

## I. THE DISCOVERY OF ELECTRIC FISHES

Fishes are the only animals which are sometimes equipped with organs specially adapted to generate an electric discharge. Yet within the Chondrichthyes and Osteichthyes, electric organs appear in a number of wholly unrelated families, occupying a wide variety of environments and must have been evolved independently at least six times. Two of the fishes capable of producing relatively large shocks—a marine elasmobranch, *Torpedo* spp. (the electric ray, νάρκη, of the Greeks), and a freshwater teleost, *Malapterurus electricus* (the electric catfish)—were well known to the ancient inhabitants of the Mediterranean shores, who figured them in paintings and wrote accounts of their application for therapeutic purposes. The Egyptians may also have been familiar with *Mormyrus* spp. of the Nile, whose discharge, although weaker, is nevertheless perceptible to the touch. The credit for first noticing the similarity between the sensation caused by an electric fish (*Malapterurus*) and the discharge of the newly discovered Leyden jar seems to be due to Adanson (1757); a more detailed comparison was made for *Torpedo* by Walsh (1773). At approximately the same time, the attention of scientists was drawn to the powerful discharge of *Electrophorus electricus* (formerly named *Gymnotus electricus*), the electric eel of South America, and the electrical nature of the phenomenon was soon recognized (Williamson, 1775). The list of electric fishes was further extended in the 19th century by the identification, on purely anatomical grounds, of "pseudo-electric" organs in the tails of various rays and skates (Stark, 1844) and of *Gymnarchus niloticus* (Erdl, 1847). With the availability of improved methods for detecting their weak discharges, these have for some time been regarded as true electric organs. Similar small organs are found in the tails of other species of gymnotid eels (Ellis, 1913) and of several mormyrids (Ogneff, 1898), and the discharges of a few of these species have recently been recorded (Lissmann, 1951; Coates, 1954). Finally, the occurrence of a moderately strong electric organ in a marine teleost, *Astroscopus guttatus* (the American stargazer), was described by Dahlgren in 1906.

The wide distribution of the various kinds of electric fish is shown in Table I. There is considerable variation in the number of electric species in the individual groups. Thus it is possible that every one of the numerous species of gymnotids and mormyrids may have electric organs, although relatively few have been examined to verify this. In view of the evident success of these groups, it would be interesting to know whether there are electric organs in any of the fishes occupying similar habitats in Asian waters, but none have ever been described. Among both the catfishes and the stargazers there is only a single electric genus out of many others. The two elasmobranch families are rather large ones, each containing a number of species; again, all those which have been suitably examined appear to have electric organs.

TABLE I

Distribution of Electric Fishes

| Group | Genera | Distribution |
|---|---|---|
| Teleosts | | |
| Mormyrids | *Gymnarchus* | Fresh waters of Africa, north of Tropic of Capricorn |
| | *Mormyrus* and others | Fresh waters of tropical Africa |
| Gymnotids | *Electrophorus* | Amazon, Orinoco, and the Guianas |
| | *Gymnotus* and others | Fresh waters of Central and South America |
| Catfishes | *Malapterurus* | Nile and fresh waters of tropical Africa |
| Stargazers | *Astroscopus* | Salt waters off Atlantic and Pacific coasts of southern North America |
| Elasmobranchs | | |
| Electric rays | *Torpedo, Narcine,* and others | All oceans, including Mediterranean Sea |
| Rays and skates | *Raia* | All temperate seas |

## II. THE ANATOMY OF THE ELECTRIC ORGAN

The common feature shared by all electric organs is that they are built up from a large number of disc-like cells called electroplaxes or electroplates, arranged in a more or less orderly fashion so that, in a given species, all face in the same direction, and thus give additive effects. Each electroplate is embedded in a jelly-like extracellular material, and enclosed within a compartment of connective tissue. Nerves and blood vessels enter each compartment, and the nerves are distributed to one face of the electroplate, while the blood vessels branch to form a network of capillaries in the jelly layer. The electroplates are single cells, although, like muscle fibers, they are multinucleate, the nuclei generally being located just beneath the two surfaces. The cytoplasm of the electroplates is relatively transparent, so that in all electric fishes the electric organs form clear gelatinous masses which can readily be distinguished from the surrounding muscle.

There is not space here to describe in detail the structure of individual electric organs, and the points mentioned must be restricted to those most obviously related to function. The best survey in English of the anatomy of electric organs is that of Biedermann (1898), while there are a number of German monographs on the subject (e.g., Fritsch, 1887, 1890). In the mormyrids, gymnotids (except *Electrophorus*), and rays, the electric organs are long spindle-shaped structures lying parallel to the spinal cord in the tail of the fish, replacing certain caudal muscles. There are altogether four organs on each side in *Gymnarchus*, two in the other mormyrids, and one on each side in the gymnotids and rays. The electroplates are arranged in columns, with their innervated sides facing forwards in the rays, and backwards in the mormyrids and gymnotids (see Table II). Each of these electric organs is fairly small, containing only one to a dozen columns, and longitudinally there are quite wide gaps between the electroplates. A spacing of the electroplates up to 2 mm. apart is also a characteristic of the organ of Sachs in *Electrophorus*, this being the most posterior (Fig. 1) of the three pairs of much larger electric organs which occupy the greater part of its body volume, forming over half its total mass. In the main organ of *Electrophorus* the electroplates are considerably closer together, there being as many as 100 per centimeter at its anterior end. In *Torpedo* and its relatives the electric organs form two large flat kidney-shaped masses on either side of the mid-line, the horizontal electroplates being packed very tightly indeed into hexagonal columns. It has often been pointed out that the shape of this electric organ and the organization of the electroplates in parallel is probably part of an adaptation which matches it better to the low electrical resistance encountered by a saltwater fish than would the series arrangement found in *Electrophorus* and other fresh-water species (see Table II). Another marine fish, *Astroscopus*, also has horizontal electroplates, arranged in close layers with no regular division into columns, filling two wells in the skull, one behind each eye (Dahlgren, 1927).

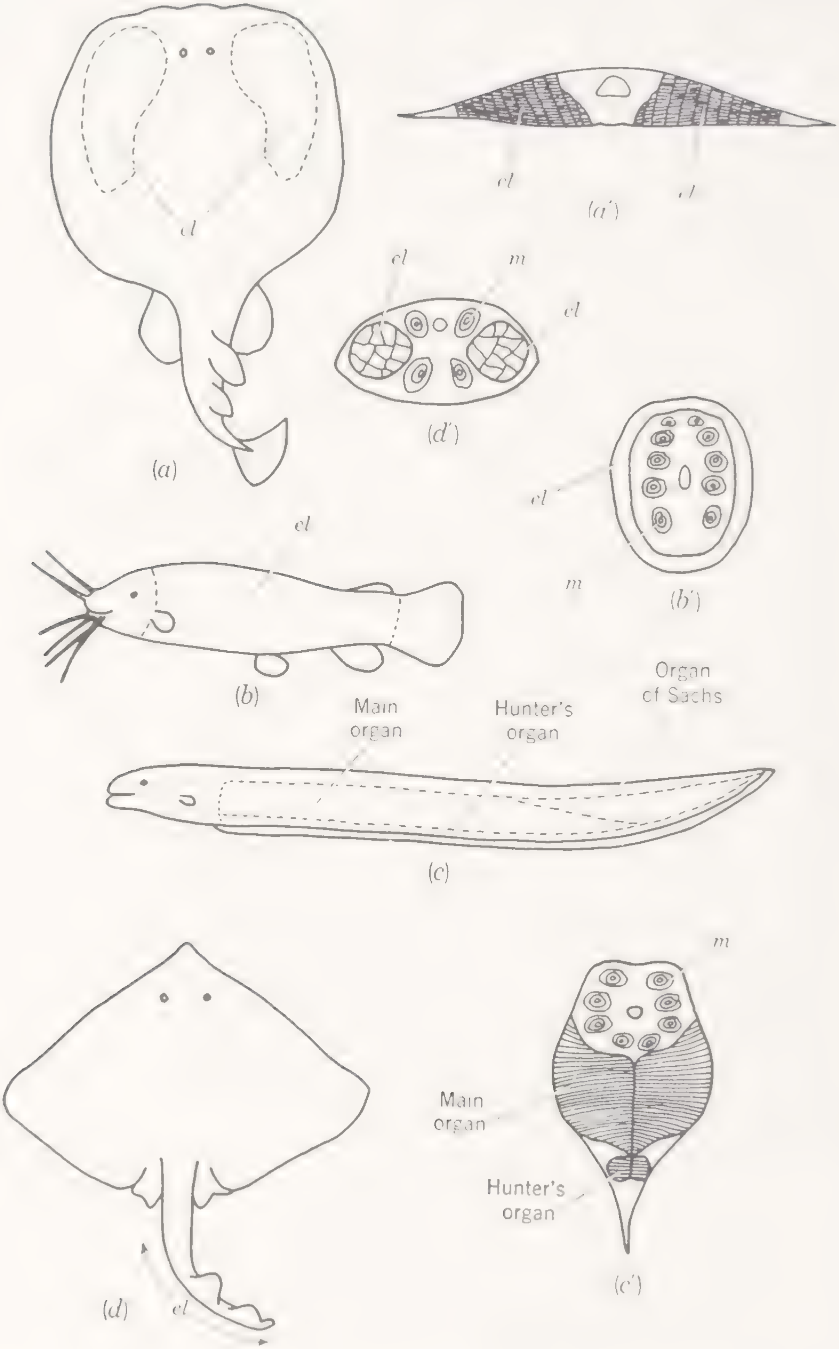

FIG. 1. Diagrams (not to scale) showing the anatomy of some electric fishes. *a*, *a′*: *Torpedo* and transverse section through its electric organs. *b*, *b′*: *Malapterurus* and transverse section. *c*, *c′*: *Electrophorus* and transverse section through main organ. *d*, *d′*: *Raia* and transverse section through its tail. m, muscle bundles; el, electric organs.

The motor nerves connected to the electric organ are most obvious in the case of *Torpedo*, where there are four large nerve trunks running from the prominent electric lobe of the brain, which is filled with unusually large motoneurones. In some living specimens of *Narcine brasiliensis* dissected by the writer, the electric lobes were colored a vivid yellow, in surprising contrast to the normal appearance of the rest of the central nervous system. On reaching the electric organ, the nerves branch, and are distributed in a very orderly way to the apices of the hexagonal electroplates. In *Electrophorus* the electric nerves arise from very large motoneurones in the spinal cord, whose processes emerge from each segment along with the ventral motor roots to the muscles, and pass to that part of the electric organ at the same level. In *Gymnarchus*, *Mormyrus* and *Raia* the innervation of the electric organ is similar to that in *Electrophorus*. In *Astroscopus* the electric nerve forms the greater part of the IIIrd cranial nerve; again the fibers arise from particularly large nerve cells in the motor nucleus (White, 1918).

The electric organ of *Malapterurus* is unique in several respects. Instead of replacing some part of the body musculature, as in all other electric fishes, it is located in the skin, where it forms a thick semitransparent jacket, loosely covering nearly the entire length of the fish. The organ is divided up in a somewhat irregular fashion by septa of connective tissue, making numerous lozenge-shaped compartments dovetailed into one another. These contain the electroplates, whose nervous faces are on the posterior sides. Their innervation is another most remarkable feature, all the electroplates in each half of the organ being connected to the processes of a single nerve cell (200 $\mu$ in diameter) situated in the gray matter of the spinal cord between the levels of the first and second spinal nerve roots. The single medullated axon arising from each cell is initially over 1 mm. in over-all diameter, being invested with multiple concentric layers of connective tissue; it ultimately branches many hundred thousand times.

One of the most striking histological characteristics of most electroplates is the considerable folding and convolution of one or both surfaces (see Dahlgren and Kepner, 1908). As a rule the face on which the nerve terminates is smooth compared with the opposite, non-nervous face, which is often studded with huge papillae. These were originally thought to have the object of increasing the area in contact with the network of capillaries in the jelly layer, and the non-nervous face was termed the "nutritive" layer, as opposed to the nervous "electric" layer. It seems more likely, however, that the papillae represent an adaptation which improves the efficacy of the discharge by lowering the effective internal resistance of the electroplates through an increase in total membrane area. Certainly in *Electrophorus*, the only species in which the resistances of the two faces have been measured separately, that of the non-nervous face is exceedingly small (Keynes and Martins-Ferreira, 1953). Another important structural question is the nature of the connection between the nerve and the electroplate. In *Raia* and *Torpedo* the nerves branch and rebranch until the points at which they are in contact with the electroplate extend over almost the whole of its surface. The area of contact is appreciably less in *Electrophorus*, where the nerves terminate on the tips of a number of short papillae. In *Mormyrus* there are still fewer contacts and this species has the peculiarity that the nerve sometimes passes through the center of the electroplate, before branching to make connection with its other side. In *Malapterurus*, contact is reduced to a single point, at the extreme tip of a complicated invagination in the center of each electroplate.

In one of the gymnotids, *Steatogenys elegans*, Lowrey (1913) has described an organ which, if it is indeed electric, occupies a different position from all others. This fish has peculiar submental filaments, cylindrical structures running in grooves beneath the dermis from the anterior margin of the lower jaw up to the bases of the pectoral fins. In cross-section each filament is seen to consist of some forty parallel discs, each with its own nerve and blood supply, and with an appearance undoubtedly very like that of an electroplate. Unhappily the fish is rather rare, and there is so far no evidence other than the anatomical description to support the view that its submental filaments are genuine electric organs.

## III. THE ORIGIN AND DEVELOPMENT OF ELECTRIC ORGANS

In all electric fishes except *Malapterurus* it has long been clear, if only from comparisons with the anatomy of related nonelectric genera, that the electric organ is derived from muscle. In *Malapterurus* the electric organ is thought to be derived from glandular tissue, largely because of its situation in the skin of the fish, and because of the absence of an axillary gland, present in all the other catfishes. It is obviously interesting to enquire into the embryological details of the conversion of muscle fibers and gland cells into electroplates.

The best known studies of the development of an electroplate are those of Ewart (1892) for *Raia batis*. He observed that in the youngest embryos the position later occupied by the electric organ was filled with muscle fibers not visibly different from those around them. In 7-cm. embryos the anterior ends of the fibers began to swell, and in 11-cm. embryos the fibers had become club-shaped; the innervation was now concentrated at the upper end of the club, due to become the nervous face of the electroplate. In later embryos the muscle tail on the posterior side gradually dwindled away, forming the non-nervous face at right angles to the axis of the original fiber. In *Torpedo* each electroplate originates from a single myoblast in a similar way, except that the initial thickening starts at the opposite (ultimately non-nervous) end of the cell (Fritsch, 1890). The embryology of *Gymnarchus* has been investigated by Dahlgren (1914), who found that in this species, as in other mormyrids, the electroplates were formed from the fusion of as many as twenty muscle cells. Lastly, White (1918) described the development of the electric organ in *Astroscopus*, which proved to be another species where the electroplates arise from single myoblasts. Dahlgren (1927) gives a fascinating account of his search for suitable material for White's researches.

While all this work confirms the muscular origin of most electric organs, it throws disappointingly little light on the question of the discharge mechanism, chiefly because there is inadequate information about the exact stages in the formation of the nerve–electroplate junctions. It is also particularly regrettable that virtually nothing is known about the breeding habits and hence the embryology of two vital species, *Electrophorus*, for which the electrophysiological information is most abundant, and *Malapterurus*, whose electric organ has so often to be listed as exceptional.

## IV. THE CHARACTERISTICS OF THE ELECTRIC DISCHARGE

The discharge of an electric organ takes the form of a series of brief pulses of current flow, whose polarity, grouping, duration, and size vary considerably from one species to another. It is hard to give exact figures for the absolute voltages produced, because these depend to a great extent on the recording conditions and are several times larger when the fish is in air than when it is in its natural element, with the low electrical resistance of the water acting in some degree as a short circuit. The values in Table II are therefore very approximate, and would probably cover a smaller range were figures available for the size of the discharge given by *Gymnarchus* in air, since the 0.03 volts recorded from it in water is unlikely to represent fairly its true capabilities. Although no precise voltages have yet been published for the mormyrids and smaller gymnotids, it is clear that a rough division can be made between those electric fishes with powerful discharges (*Electrophorus*, *Malapterurus*, *Torpedo*, and *Astroscopus*) and those with weak ones (*Gymnarchus* and other mormyrids, gymnotids, and *Raia*). As will be seen later, there is a corresponding difference in the function served by the discharge. In

TABLE II

CHARACTERISTICS OF ELECTRIC ORGANS

| Species | Orientation of Nervous Face | Polarity of Discharge | Total Number of Electroplates | | Origin | Maximum Recorded Size of Discharge (volts) |
|---|---|---|---|---|---|---|
| | | | In Series | In Parallel | | |
| *Mormyrus oxyrhynchus* [a] | Posterior | Head + ve | 160 | 4 × 1 | Caudal muscles | ? |
| *Gymnarchus niloticus* [b] | Posterior | Head + ve | 140 | 8 × 1 | Caudal muscles | 0.03 |
| *Electrophorus electricus* [c] | Posterior | Head + ve | 6000 | 2 × 35 | Caudal muscles | 550 |
| *Gymnotus carapo* [d] | Posterior | Head + ve | ? | 2 × 5 | Caudal muscles | 0.3 |
| *Malapterurus electricus* [e] | Posterior | Tail + ve | 1600 | 3000 | Gland in dermis | 350 |
| *Astroscopus guttatus* [f] | Dorsal | Ventral side + ve | 200 | 2 × 20 | Eye muscles | 50 |
| *Torpedo marmorata* [g] | Ventral | Dorsal side + ve | 400 | 2 ×550 | Branchial muscles | 45 |
| *Torpedo nobiliana* [h] | Ventral | Dorsal side + ve | ? | 2 × 1050 | Branchial muscles | 220 |
| *Narcine brasiliensis* [i] | Ventral | Dorsal side + ve | 300 | 2 × 380 | Branchial muscles | 37 |
| *Raia clavata* [j] | Anterior | Tail + ve | 200 | 2 × 12 | Caudal muscles | 4 |

[a] From Ogneff (1898).

[b] From Erdl (1847), Lissmann (1951).

[c] From Albe-Fessard (1950a).

[d] From Shaffer (1917), Coates *et al.* (1954).

[e] From Fritsch (1887), Remmler (1930).

[f] From Dahlgren (1927).

[g] From Albe-Fessard (1950a).

[h] From Fritsch (1890), Coates and Cox (1942).

[i] From Cox and Breder (1943).

[j] From Brock *et al.* (1953).

*Electrophorus* the electric organ appears to have a dual nature, and this fish ought perhaps to be listed in both groups.

In all the freshwater species, the potential difference during the discharge arises between the head and tail of the fish, although as Table II shows, the polarity is not the same in every case. In *Raia* also, the voltage gradient is along the spinal cord, but in the other marine species the electroplates are arranged horizontally so that the voltage drop occurs between the dorsal and ventral surfaces of the fish.

In the discharge of the powerful electric organs, the pulses typically occur in small groups, as a reflex response under the control of the central nervous system. In *Electrophorus* (see Fig. 2) the groups of four large pulses, usually preceded by a smaller pulse, may be repeated several times per second. In addition, this fish can produce a continuous series of much smaller pulses (of the order of one-tenth as large), at a variable repetition rate between about 1 and 50 per second (Keynes, 1952; Coates, 1954). The discharge of *Torpedo marmorata* consists of similar groups of somewhat longer pulses, but here the preparatory small pulse, and the continuous low-frequency discharge, are not observed. In 1914 Fuji (using a Duddell oscillograph) recorded the natural discharge of another electric ray, *Astrape japonica*, as pairs of pulses, while *Torpedo nobiliana* (= *T. occidentalis*) gives an average of a dozen pulses in each group (Coates and Cox, 1942). *Malapterurus* produces trains of ten or more rather brief pulses (duration about 3 milliseconds at 30° C.), spaced very close together (Koike, 1910). The discharge of *Astroscopus* has apparently still to be investigated with an oscilloscope.

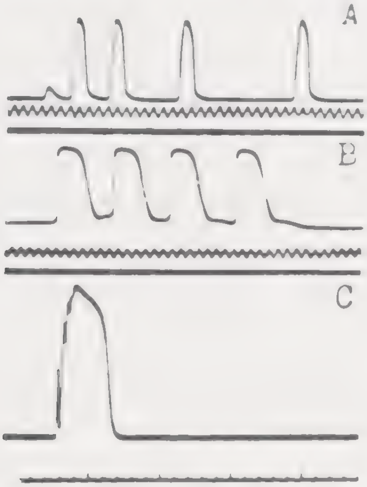

FIG. 2. The natural discharges of: A, *Electrophorus electricus;* B, *Torpedo marmorata;* C, *Raia clavata.* Time markers are milliseconds for A and B, ⅕ second for C (from Albe-Fessard, 1950a).

*Raia* also produces a reflex response, this being a single long pulse (Fig. 2) resulting from the asynchronous discharge of a number of electroplates (Albe-Fessard and Couceiro, 1950). But in the mormyrids and in the gymnotids other than *Electrophorus*, the weak electric organs generate a continuous series of pulses, either [in the mormyrids other than *Gymnarchus* and in *Gymnotus carapo* (Lissmann, unpublished data)] at a rather variable rate, or, in a few species, at a frequency which is independent of the state of activity of the fish at any given moment and varies only with temperature. Thus in *Gymnarchus*, the repetition rate is 318 per second at 28° C. (Lissmann, 1951), while in the gymnotids *Eigenmannia virescens*, *Gymnorhamphichthys postumus*, and *Gymnotus carapo*, it has been recorded by Coates, Altamirano, and Grundfest (1954) as 300, 100, and 65 per second, respectively, at 27° C.

## V. THE DISCHARGE OF A SINGLE ELECTROPLATE

It will already be apparent that there was considerable research done during the 19th century on the anatomy of electric organs. Great efforts were also devoted to studies of their physiology, but owing to the inadequacy of the techniques then available for examining electrical phenomena, relatively few results of comparably permanent value were obtained. Perhaps the most important generalization to emerge was Pacini's Law, which states that, with the usual exception of *Malapterurus*, the nervous face of the electroplate becomes electrically negative to the non-nervous face during the discharge, whatever the orientation of the electric organ as a whole. The truth of Pacini's observation may be judged from Table II. But in the articles of Biedermann (1898) and Gotch (1900), no good explanation was given for this behavior, nor were any useful suggestions made as to the origin of the potential changes within living cells.

The situation was altered shortly afterwards by the brilliant work of Bernstein (1902) and Bernstein and Tschermak (1906), who applied the physico-chemical theories of Nernst and others. These led to the idea that there was a semi-permanent polarization of both faces of the electroplate, the inside being negative, arising from differences in electrolyte concentrations between the cell contents and the extracellular medium, and that the discharge was produced by a temporary collapse of the selective permeability of the nervous face (Bernstein, 1912). Yet although Du Bois-Reymond (1887) had shown that the resting organ of *Torpedo* could generate a sustained electric current, all efforts to provide really satisfactory evidence for or against Bernstein's hypothesis proved fruitless until the recent introduction of reliable methods for direct determination of the potential difference across cell membranes. The application of microelectrode techniques to electroplates from the organ of Sachs in *Electrophorus* at once showed that Bernstein was basically right, but that at the peak of the discharge the membrane potential across the nervous face is substantially reversed, rather than merely falling towards zero (Keynes and Martins-Ferreira, 1953; Altamirano, Coates and Grundfest, 1955). The potential across the non-nervous face is virtually unchanged during the spike. The way in which a column of electroplates is thus enabled to discharge additively is illustrated in Fig. 3, which also shows the absolute sizes of the membrane potentials.

The evidence in both the papers quoted above strongly supports the view that in *Electrophorus* the electroplate should be regarded as a modified muscle fiber, with a normal muscle membrane on its nervous face, triggered by the release of a chemical transmitter at nerve endings occupying only a small fraction of its total area, and on the opposite side an inexcitable membrane specially adapted in having an exceptionally low electrical resistance. The reversal of potential during the spike and its behavior on variation of the external sodium and potassium concentrations, the ability of the nervous face to conduct a propagated impulse, and the fact that it can be stimulated directly with almost no delay by a small depolarization (Albe-Fessard, Chagas, and Martins-Ferreira, 1951) are typical of the properties of a normal excitable membrane. On the other hand, the work of Chagas and Albe-Fessard (1954) on the action of curare and related drugs, which block indirect (delayed)

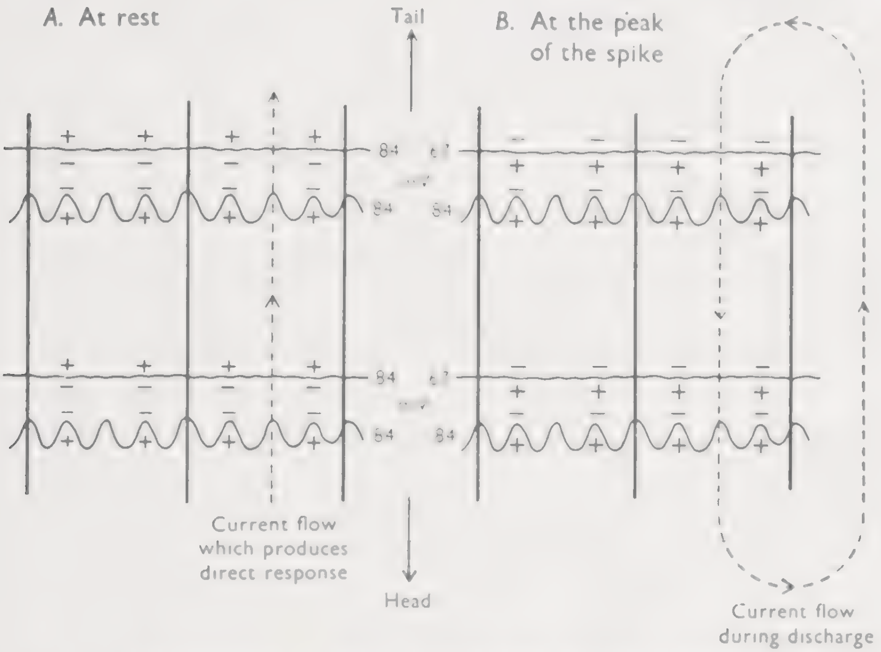
A. At rest
Tail
B. At the peak of the spike
84
67
mV
84
84
84
67
mV
84
84
Current flow which produces direct response
Head
Current flow during discharge

FIG. 3. The mechanism of additive discharge in the electroplates of the organ of Sachs in *Electrophorus*. At rest (A) there is no net potential across the electroplates, but at the peak of the spike (B) all the potentials are in series, and the head of the eel becomes positive with respect to its tail. From Keynes and Martins-Ferreira (1953); Altamirano *et al.* (1955) found somewhat smaller average voltages.

excitation via the nerve endings without impairing direct excitability, suggests that acetylcholine (ACh) acts as a link at the nerve–electroplate junctions. It is also difficult to account in any other way for the observation of Chagas (1952) that the ACh content of the main organ, never particularly high, was markedly reduced after indirect stimulation, but was very little changed after the same amount of direct stimulation. Altamirano *et al.* (1955) obtained convincing evidence for the intervention of a chemical transmitter, and demonstrated the occurrence of postsynaptic potentials across the nervous face which could be differentiated from local responses similar to those observed in muscle and nerve, but they came to the conclusion that the transmitter was not ACh. However, they had not at the time of writing published their reasons in full.

Much has been made of the finding that the voltage generated by different parts of the electric organ in *Electrophorus* is proportional to the amount of cholinesterase present (Nachmansohn, Coates, and Rothenberg, 1946), which has been taken to prove that ACh has a more universal role than that just attributed to it. Since the voltage also increases linearly with the number of electroplates in action (Cox, Coates, and Brown, 1945), the proportionality probably implies no more than that each electroplate contains (or as has been shown by Couceiro, Almeida and Freire (1953) has closely associated with its nervous face) roughly the same quantity of cholinesterase.

In the electric organs of elasmobranchs, the older view that the electroplate is a highly developed motor end-plate seems likely to be correct. In *Raia clavata*, microelectrode studies have shown that at the top of the spike there is little or no reversal of the potential across the nervous face (Brock, Eccles, and Keynes, 1953); both in this respect and in its shape the action potential bore a strong resemblance to the end-plate potential at a frog neuro-muscular junction (Fatt and Katz, 1951). In *Torpedo* the crucial evidence is still lacking, since although a resting potential of about 50 millivolts has been recorded (Fessard and Tauc, 1951), the action potential across the nervous face has not yet been observed. But the facts that in neither of these elasmobranchs can the electric organs be excited directly, but only with a delay of several milliseconds (Auger and Fessard, 1939; Fessard, 1947), and that in *Torpedo* the organ becomes totally inexcitable after curarization or degeneration of the nerve supply (Fessard, 1946) support the end-plate hypothesis. Moreover, Fessard (1952) has pointed out that histologically there is a contrast between the electroplates of *Torpedo* and *Raia*, in which nerve endings cover densely the whole of the nervous face, and those of all other electric fishes, in which the area occupied by the terminations is more or less restricted. ACh would again appear to act as a transmitter, and if the nervous face in *Torpedo* consists of virtually nothing but end-plate membrane, it is not surprising that the ACh content of the electric organ should be over ten times that in *Electrophorus*, that injected ACh should have an electrogenic effect, and that measurable quantities of ACh should be released on stimulation (Feldberg and Fessard, 1942).

Although much corroborative evidence still needs to be gathered, it can be concluded that the nervous face of the electroplate is primarily a specialized end-plate in *Torpedo* and *Raia*, but that in almost all the teleost electric organs it is more properly to be thought of as a modified muscle membrane. The polarity of the discharge will be the same in either case—and Pacini's Law will be obeyed—since the potential across the nervous face will always change in the direction of the outside becoming less positive, or even negative. It remains to account for the aberrant behavior of *Malapterurus*. A complete explanation is unlikely to be forthcoming until its electric organ has been attacked with microelectrodes, but in the meantime a suggestive clue has been provided by the observation of Lundberg (1954) that in the cells of salivary glands secretory activity is accompanied by an increase in the internal negativity. This is a potential change in the reverse direction to that occurring in active nerve and muscle so that, if other gland cells behave similarly, the failure of the electroplates of *Malapterurus* to conform with Pacini's Law would fit neatly with their supposed embryological origin from glandular tissue.

Bernstein and Tschermak (1906) argued that their thermodynamic experiments on *Torpedo* proved that the energy dissipated during the discharge was not derived immediately from a chemical reaction, but came from energy previously stored in the electroplates in the form of ionic concentration gradients. This interpretation agrees well with current views about the energetics of the action currents in nerve and muscle. On the ionic hypothesis of nervous conduction (Hodgkin, 1951), the rising phase of the spike results from an entry of $Na^+$ ions into the cell during a transient increase in the sodium permeability of the membrane, while the falling phase is brought about by a leakage of $K^+$ ions during a delayed increase in the potassium permeability. The energy source for the electric currents which flow during propagation of an impulse is thus the movement of first sodium and then potassium from strong to weak solutions. The wave of depolarization which occurs at a motor end-plate is thought to arise in a somewhat different way (Fatt and Katz, 1951), a release of ACh from the nerve ending causing a nonselective increase in the permeability of the end-plate to all the ions present; but again the chief energy source is a mixing of ionic solutions. There is, unfortunately, rather little direct evidence to prove that the discharge of electric organs depends on ionic movements similar to these. Although Overton (1902) long ago suggested that it would be instructive to observe the behavior of an electric organ deprived of sodium by immersion in an isotonic sucrose solution, the experiment remained untried until Keynes and Martins-Ferreira (1953) found that such treatment did indeed abolish its activity in a reversible manner. They also recorded resting potentials whose absolute size fits well with recent potassium analyses of the tissue (Davson and Lage, 1953). The impedance variations in *Torpedo* (Albe-Fessard, 1950b) provide another parallel with the nerve impulse. Taking these few observations with Bernstein and Tschermak's (1906) cogent arguments, and remembering the embryological origin of the electroplates, there can be little doubt that the immediate cause of the potential changes is a downhill passage of ions across the cell boundaries, presumably mediated through the operation of mechanisms like those in nerve and muscle.

A supply of energy from metabolic sources is likely to be necessary to drive secretory processes to restore the concentration gradients after activity. Thus in cephalopod nerves, recovery after stimulation has been shown to involve an extrusion of sodium coupled to an active uptake of potassium, both being dependent on metabolism (Hodgkin and Keynes, 1955). Nachmansohn *et al.* (1946) have shown that in *Electrophorus* the observed breakdown of phosphocreatine could provide enough energy for the restitution of the resting state. In subsequent publications Nachmansohn (1951, etc.) assumes that the whole of this energy is needed to resynthesize ACh broken down during the discharge. But since in *Electrophorus* ACh seems only to act as a junctional transmitter, it is improbable that its resynthesis requires more than a minor proportion of the total energy expenditure, the greater part being reserved for driving ionic pumps. This is probably true even in *Torpedo* if, as at the motor end-plate (Fatt and Katz, 1951), the amount of $ACh^+$ ions released by the nerve endings can account for only a small part of the total charge transferred through the membrane.

## VI. THE NERVOUS CONTROL OF THE ELECTRIC ORGAN

Even in those species which generate a continuous series of pulses, the electric organ is under cephalic control. Thus Coates *et al.* (1954) showed that cooling only the heads of various small gymnotids had the same slowing effect on the discharge as cooling the whole fish. In other electric fishes it has long been clear that the discharge must be controlled by the central nervous system, if only because it occurs as a reflex response to appropriate stimuli. A certain amount is known, chiefly from the anatomical side, about the motor pathway from the brain to the electric organ, but there is very little information about the sensory channels involved in eliciting the discharge. One of the few studies on this question is the recent work of Fessard and Szabo (1953) on *Torpedo*, where a nucleus of nerve cells lying in the white matter at the level of the VIth cranial nerve has been identified as a relay station between the sensory afferents and the electric lobe of the brain. But neither in this species nor in any other is it known where the integration leading to the discharge occurs, nor where its natural rhythm is determined.

An intriguing problem in this connection is that of the close synchronization of the discharge of the individual electroplates, the advantage of which in achieving large total voltages is obvious. Coates *et al.* (1940) observed with recording electrodes distributed along specimens of *Electrophorus* that each action potential traversed the electric organ with an apparent velocity of 1000 m. second or more. Since it seemed unlikely that the motor pathways leading to the organ could conduct impulses at anything approaching this speed, they suggested that there might be some form of compensation in order to allow signals to reach both ends of the organ at roughly the same moment. Albe-Fessard and Martins-Ferreira (1953) have now described in detail how it is that the discharge of the whole organ lasts only about 1.5 milliseconds longer than the discharges of the isolated electroplates, although the time for conduction along the spinal cord may be as much as 10 milliseconds. The two most important synchronizing factors are a decrease of several milliseconds in the synaptic delay at the spinal motoneurones, and a reduction of several centimeters in the lengths of the motor nerves arising from them, between the anterior and posterior parts of the fish. Two other factors—a temporal spreading of the impulses leaving the brain, and a facilitation of the posterior part of the organ by the discharge set up in the anterior part—appear to play a secondary role. It would be interesting to know how the discharge is synchronized in other electric fishes (in *Raia* it seems to be asynchronous). All that can be said at present is that in *Torpedo* the anatomical layout is such that differences in conduction time must be rather small, while in *Malapterurus* (which may grow to a length of over three feet) the mechanisms discussed for *Electrophorus* cannot very well be operative, since the whole organ is innervated by only two axons. Perhaps in this case facilitation within the organ is more effective, or, since a single shock to the nerve gives rise to a long train of pulses (Gotch, 1900), there is a large element of self-excitation once the discharge has been started.

In *Electrophorus*, the degree to which facilitation is effective in different parts of the electric organ may have functional importance. According to Albe-Fessard and Chagas (1954) the discharge of the main organ is greatly facilitated by the summation of successive nerve impulses, but that of the organ of Sachs is not, and it responds "coup pour coup" to nerve stimulation. Under resting conditions impulses reach the electric organ at a frequency of no more than 1 or 2 per second, and only the organ of Sachs responds, producing the "small pulses." During reflex stimulation of the fish, the nervous rhythm is rapidly accelerated until the point is reached where the main organ is activated, thanks to the facilitation effect. The switch-over from the small pulses to the full-size ones thus seems to be largely automatic.

## VII. THE BIOLOGICAL FUNCTION OF THE ELECTRIC ORGAN

There is no doubt that those species capable of really powerful discharges use them both for offensive and for defensive purposes. In the words of Gotch (1900), "The *Gymnotus*, kept in a tank, will discharge when small live fish are placed in the water. The discharge stuns a certain number of the fish, and the sluggish eel is thus able to devour them at his leisure." Oddly enough, it is only recently that *Torpedo* has been watched in the act of stunning its prey, although the discovery of quite large fishes in the stomachs of several specimens could only be explained on the assumption that they had been electrocuted before being swallowed (Bigelow and Schroeder, 1953). However, Wilson (1953) persuaded *Torpedo nobiliana* in the Plymouth aquarium to pounce on dead fish towed through the water on wires, and gave a graphic account of their mode of attack. He also described their use of their discharge as a protection against the attentions of other marine creatures in the tank. Since the stomach contents in some of the smaller electric rays, like *Narcine brasiliensis*, consist mostly of small worms and crustacea, which would hardly need to be electrocuted, it seems possible that the primary role of the discharge may sometimes be defensive, although if this is the case, specimens kept in aquaria do not necessarily make effective use of their powers (Bigelow and Schroeder, 1953). It is clear from some of the accounts of the habits of *Malapterurus* (Du Bois-Reymond, 1887) and of *Astroscopus* (Dahlgren, 1927) that the discharge may be used to stun other fishes in a tank, but it does not seem to have been established whether these species are accustomed to use their discharge in normal feeding, or whether it is reserved for defense. The readiness with which all these electric fishes discharge when touched or alarmed in any way suggests that, however much or little they use their electric organs offensively, they are always ready to put them to defensive use as well.

The function of the weak electric organs long remained a puzzle. Then it was suggested by Coates (1950) that the small low-frequency discharges apparently emitted by the organ of Sachs in *Electrophorus* might form part of a direction-finding system, and this idea received strong support when Lissmann (1951) found that *Gymnarchus* was certainly able to discriminate between conductors and nonconductors placed in the water beside it. Tests similar to Lissmann's were later made on *Electrophorus* (Keynes, 1952). Coates *et al.* (1954) have now recorded continuous small discharges in several other gymnotids, and it seems certain that both in this group and in the mormyrids, the electric organ acts as a transmitter for a direction-finding device which depends on the detection of changes in the pattern of current flow in the water around the fish. As yet almost nothing is known about the receiving end of the system, and apart from a statement of Coates (1954) that painting the head of an electric eel with insulating lacquer disturbs its sense of direction, even the location of the receptors is a mystery. But many kinds of fish are highly sensitive to small electric currents, and there is some evidence that the lateral line organs may serve as current detectors (Regnart, 1931). It would also be interesting to know whether the enormous elaboration of the cerebellum and lateralis nerves in the mormyrids (van der Sprenkel, 1915) is somehow related to their direction-finding mechanism. Investigation of this whole problem offers an exciting new field in sensory physiology.

Lissmann (1951) pointed out that his observations helped towards explaining the evolution of the large electric organs which had troubled Darwin (1859) because of the seeming absence of any transitional step. It is not impossible to imagine how *Electrophorus*, already having a small organ for direction-finding, found more lethal applications for its discharge, until it ultimately became the remarkable example of specialization that we see today. But it is still necessary to account for the initial evolution of small electric organs in the mormyrids and gymnotids, as well as that of the relatively powerful organs in *Malapterurus* and some of the marine species. There is no evidence that the organs in the torpedos and rays ever serve for direction-finding, although fish like the blind torpedos, *Benthobatis* and *Typhlonarke*, and those rays which live in very deep water (Bigelow and Schroeder, 1953), could doubtless make good use of such a faculty. Moreover, any electric direction-finding system would have a very restricted range in sea water, because of its low electrical resistance. As far as the rays are concerned, it is hard to see what useful purpose the electric organ can serve, since its discharge seems to be provoked only by vigorously prodding its back and wings, and yet is rather weak to be an effective deterrent to enemies, unless it is successful in mimicking the stronger discharge of *Torpedo*.

Another possibility (suggested by the Editor) is that the electric organs may provide means for species, perhaps even for sex, recognition. Most electric fishes live in situations where visual recognition, except through luminous organs, can be of little use; some other fishes in these habitats make characteristic noises. The electric signals vary between species and, if the fishes have sense organs capable of detecting the patterns of the discharges, these could be used for the identification and location of other individuals of the same species. Such a function for electric organs could explain their appearance in marine as well as in freshwater fishes.

## VIII. THE IMMUNITY OF ELECTRIC FISHES TO THEIR OWN DISCHARGE

One final question which has bothered many physiologists, including the distinguished team of Faraday and Du Bois-Reymond (see Du Bois-Reymond, 1887), is to explain how those electric fishes with powerful discharges avoid electrocuting themselves. Part of the answer is clearly that the anatomical arrangement is such that the lines of current flow inside the fish tend to cross other excitable structures at right angles, thus minimizing their stimulatory effect. But this is certainly not the whole explanation, since electric fishes are also fairly well protected against externally applied shocks—against each other's discharges for example. It is probable both that their nervous systems have an unusual degree of insulation (in *Electrophorus* the spinal cord and swimming muscles are embedded in a thick layer of fat, which would be a good insulator), and that their nerves and muscles may even, when exposed, have an especially high threshold for excitation. However, there is very little information about the exact distribution of electric current within the body of the fish, and this is another subject which might repay further research.

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## Chapter VI

# THE LUMINOUS ORGANS OF FISHES

E. Newton Harvey

Page
I. Historical Introduction . . . . . . . . . . . . . . . . . . . 345
II. Distribution of Luminescence . . . . . . . . . . . . . . . . . 348
III. Types of Luminescence . . . . . . . . . . . . . . . . . . . 348
    A. Luminous Bacterial Symbiosis . . . . . . . . . . . . . . . . 350
        1. Macrouridae . . . . . . . . . . . . . . . . . . . . 350
        2. Gadidae . . . . . . . . . . . . . . . . . . . . . 350
        3. Monocentridae . . . . . . . . . . . . . . . . . . 350
        4. Anomalopidae . . . . . . . . . . . . . . . . . . 352
        5. Acropomatidae . . . . . . . . . . . . . . . . . . 353
        6. Leiognathidae . . . . . . . . . . . . . . . . . . 353
        7. Serranidae . . . . . . . . . . . . . . . . . . . 354
        8. Saccopharyngidae . . . . . . . . . . . . . . . . . 354
        9. Ceratioidea . . . . . . . . . . . . . . . . . . . 354
    B. Self-Luminous Photophores . . . . . . . . . . . . . . . . . 355
        1. Elasmobranchii . . . . . . . . . . . . . . . . . . 355
        2. Stomiatoidea and Myctophoidea . . . . . . . . . . . . . 356
        3. Batrachoididae . . . . . . . . . . . . . . . . . . 360
IV. Nerve and Hormone Control . . . . . . . . . . . . . . . . . 362
V. Use of the Light . . . . . . . . . . . . . . . . . . . . . 363
    References . . . . . . . . . . . . . . . . . . . . . . . 364

## I. HISTORICAL INTRODUCTION

It is extremely difficult to say who observed the first luminous fish. In Pliny's Natural History (IX, 27) there is a description of the lantern fish (*lucerna piscis*). According to the Holland translation of 1601, "it lilleth forth the tongue out of the mouth, which seemeth to flame and burne like fire, and in calme and still nights giveth light and shineth." Various commentators have considered the statement to refer to a luminous jelly-fish or to the fire-cylinder, *Pyrosoma*, abundant in the Mediterranean. Such an interpretation, rather than true luminescence of a fish, seems likely.

Most early records of fish luminescence suffer from the doubt that the light may have come from the dead animal and be due to luminous bacteria, or, if the fish was living, result from small luminous organisms in the sea water, stimulated by the moving fish and outlining its body in fire. Risso in 1810 described a luminous slime oozing from the snout of "Chimère arctique," which was probably a growth of luminous bacteria. However, his 1820 paper on the "Scopeles" from Nice contains figures of fish with the prominent skin structures which we now know as photophores. Achille Valenciennes described a "Scopele lumineux in the last volume of his "Histoire naturelle des Poissons" (Paris, 22 vols. 1828–1849).

In 1833, Anastasio Cocco described deep-sea fish from Messina and in 1838 used the expression "punti luminosi" or "lucidi" and "apparecchio lucido" for the skin structures. His figures show the photophores clearly and he worked in a region where light from the fish could have been observed. Franchesco Orioli wrote "De pesci e del mare che rilucono nella oscurita" in 1850, and in 1854 J. T. Reinhardt reported the greenish luminescence of *Astronesthes* photophores during a voyage to Brazil. In the meantime an account of the first luminous shark on record was published in 1840 in F. D. Bennett's book describing a whaling voyage around the world in 1833–1836.

The great histologist, Rudolf Albert Kölliker (1817–1905) in 1853 thought the skin spots of deep sea fish were sense organs, while Rudolph Leuckard (1822–1898) in 1865 regarded them as accessory eyes (Nebenaugen). They were called modified eyes in a long paper by Ussow as late as 1879.

Space does not permit a record of the controversy over the function of the "pearly spots" in the skin of fish. The discussion continued until after 1879, when Franz Leydig designated the "Nebenaugen" of *Chauliodus* as probable luminous organs. In his book "Die Augenahnlich Organe der Fische," published in 1881, he became convinced that their function was photogenic. It was the era of deep-sea exploration, particularly the voyage of the Challenger in 1873–1876 that made scientists realize how large the deep-sea fish population is, and that many of them are luminous.

Study of the histology of fish light organs was pioneered by C. Emery in 1884 and R. von Lendenfeld in 1887, followed by a host of workers at the turn of the century. The enthusiasm for discovery of light organs in fish became so great that many structures described as photogenic are very dubious, and quite a number of species designated luminous are doubtful (see Harvey, 1952).

In recent times it has been discovered that a number of fishes are not self-luminous but possess special organs in which symbiotic luminous bacteria are cultivated. These bacteria are always present and the light organ is a permanent structure, often of complicated make up, with reflectors and devices for shutting off the light. The first inkling of the presence of bacteria was contained in a short note of B. Osorio (1912) regarding the macrourid fish, *Malacocephalus laevis*, which when disturbed, secretes the luminous bacterial mass through a duct. The second observation was made by the author (Harvey, 1923) for the fishes, *Anomalops* and *Photoblepharon*, of the Banda Islands, Indonesia. These forms possess a large permanent light organ containing luminous bacteria under the eye. The bacteria are not extruded but remain in place in elongate cells richly supplied with blood vessels. Although the fish have been known since the observations of Peter Boddaert in 1781, the first suggestion that the peculiar eye structure was luminous was made by A. Günther in 1887, and the light was later observed by A. G. Vordermann in 1900, without his realizing that bacteria were present. Many other groups of fish are now known to possess symbiotic luminous bacterial lights.

In the present account only the fish whose luminescence has been seen and whose luminosity, whether from symbiotic bacteria or photogenic cells of the fish, is beyond question, will be considered.

Study of the physiology of fish luminescence may be said to date from the end of the nineteenth century when Greene (1899) tested electrical and mechanical stimulation of the California toadfish, *Porichthys*, finding that it was rather difficult to excite light production in this way. In an appendix to the morphological monograph of Johann (1899), Beer reported that a Naples elasmobranch, *Spinax*, would also luminesce on electrical stimulation. Later, Greene and Greene (1924) discovered that the California toadfish would readily respond by intense luminescence of the photophores to the injection of adrenaline and this opened the way to the possibility of hormonal control.

However, physiological studies have not been extensive, chiefly because deep-sea fish are hard to obtain and do not live long when caught. The author is unaware of any records of photophore luminescence response in which light intensity has been determined as a function of time. The biochemistry of fish luminescence has been almost completely neglected, chiefly as a result of the scarcity of material to work with. Haneda and Harvey (1954) could not demonstrate a luciferin-luciferase reaction with *Diaphus* or *Polyipnus* and adenosine triphosphate did not appear to be essential for luminescence.

The intensity of the light organ of *Photoblepharon* has been measured by Steche (1909) as 0.0024 MK (candle). No spectral distribution curves of the light of fish have been obtained, but Takase (1938, 1939) has studied the spectrum of symbiotic bacteria from the light organ of the deep-sea fish, *Coelorhynchus*, finding a maximum at 5200 Å in 0.5 *M* cane sugar solution and at 4700 Å in 0.8 *M* NaCl. Akabane's (1938) curve was somewhat more symmetrical. Haneda (1938) placed the maximum for bacteria from *Malacocephalus laevis* at 5100 A. These spectral distributions are similar to those obtained from ordinary saprophytic luminous bacteria.

## II. DISTRIBUTION OF LUMINESCENCE

The various headings of the next section indicate the groups of fish whose light emitting ability has been established beyond question. Some are surface forms and some are deep-sea forms which come to the surface to breed, such as the California toadfish of the Haplodoci, family Batrachoididae. The great majority are bathypelagic, living at a low temperature and high pressure, and are world wide in distribution. Many examples are to be found among the angler fishes, the Ceratioidea, with about a dozen families containing luminous species, as well as among the "lantern fishes" of the Stomiatoidea (about 10 families with luminous species) and the Myctophoidea, with four families of which the Myctophidae (or Scopelidae) are particularly noted for luminescence. Some additional teleosts of doubtful luminosity have been discussed by Harvey (1952).

Bathypelagic fish, including the Ceratiodea, live at medium depths (500–2500 meters) in the twilight zone or below, rather than at the bottom. They sometimes migrate to the surface at night. Because of the uniform environmental conditions in the deep sea, one species may be found in widely separated oceans. Haffner (1952) has made a careful study of the zoogeography of various species and subspecies of *Chauliodus*, finding "that each population is restricted to certain water masses identified by their temperature-salinity characteristics." Various estimates have placed the proportion of luminous species among deep-sea forms caught by the Valdivia expedition at 6 to 11% for those living below the 400-meter line (Brauer, 1906, 1908). Weill (1938) concluded that number of individual fish was a better test of abundance and placed the luminous individuals of the Valdivia expedition at 78 to 88% of all specimens taken. Based on extensive collection in a local region south of Bermuda, Beebe (1937) found that the luminous individuals caught below 300 fathoms were 95% of the total catch, with the genus *Cyclothone* most abundant.

## III. TYPES OF LUMINESCENCE

There can be no doubt that a primary classification of luminous fish should be based on those which are self-luminous, and those whose light comes from symbiotic luminous bacteria. Such a division has been followed below, although the evidence for bacterial luminescence has not been thoroughly tested in certain groups, i.e., symbiotic luminous bacteria have not been grown in the case of the Serranidae, the Saccopharangidae and the Ceratioidea. Their general behavior makes bacterial light seem likely. An important characteristic of bacterial luminescence is the fact that light intensity remains constant over long time periods so that accessory devices are necessary to turn the bacterial light on and off. Moveable opaque screens, rotation of the light organ, or expansion and contraction of chromatophores near the symbiotic luminous bacteria have been developed.

Haneda (1950) has referred to certain types of bacterial light organs where the luminous bacteria live in the cavity of a glandular structure deep in the body and the light shines through the muscle tissue as "indirect emission organs." Such a condition is to be found in the families Acropomatidae, Leiognathidae, and Serranidae.

Among the fish with self-luminous skin organs, the presence or absence of symbiotic luminous bacteria has been carefully tested by Haneda (1952) only in the case of *Yarella* of the Gonostomatidae and *Polyipnus* of the Sternoptychidae. None were found in these two genera. In elasmobranchs, Batrachoididae, stomiatoid and myctophid fish it seems most unlikely that luminous bacteria are responsible for the light. The organs are always under control and in many cases can flash rather rapidly.

A classification of luminous fish based on the position of the light organs is hardly feasible because photophores occur in every conceivable position, although they are usually ventral or lateral rather than dorsal. They occur on the ends of illicia, or barbels, or long tentacles, as well as deep within the body near the digestive tract. Mostly they form rows or patterns on the skin, usually with a large photophore near the eye, or on the eyeball, or sometimes photophores on the tongue, as in *Neoscopelus* (Kuwabara, 1954). Even the teeth have been described as luminous, although the source of the light requires investigation.

The meaning of one or more light organs near the eye (cheek or suborbital photophores) is not clear. Frequently they respond to stimulation in a different way from other organs and may flash intermittently, as in *Echiostoma*. The same problem is presented by other deep-sea luminous animals, for example, squid, which are particularly characterized by light organs on the eyeball, and deep-sea shrimps with photophores near the eye.

A classification of luminous fish depending on the structures of the light organ might be based on the presence or absence of openings to the outside, on the presence or absence of a reflector and other accessory structures, etc. However, it is often observed that the same fish may possess photophores of very different types, and in a single family they may vary from the most complicated lanterns, to relatively simple groups of photogenic cells. Usually the light organs of fish with symbiotic luminous bacteria are open to the sea water, either by means of a long duct through which bacteria can be squeezed by muscle contraction (Macrouridae) or by smaller pores from which the bacteria never emerge in sufficient number to appear as an external luminous secretion (Anomalopidae). There is the widest variety of histological structure, which cannot be described here.

## A. Luminous Bacterial Symbiosis

### 1. Macrouridae

The best-known examples of these fish are *Malacocephalus laevis* and *Coelorhynchus coelorhynchus*, with a large internal luminous gland opening by a duct to the ventral wall of the fish just anterior to the anus (Fig. 1). A secretion of "particles" (Hickling, 1925-26, 1931), actually luminous bacteria which have been cultured (Haneda, 1938), can be squeezed out.

The gland is richly supplied by blood vessels but there appears to be no special nerve supply, certainly not from the rectum. However, there is "a network of nerve cells in the connective tissue capsule of the gland, from which fibers pass into the folds of tissue which project into the lumen; this network seems to be supplied by nerves arising from the dermis below the gland."

According to Haneda (1938, 1951) a faint light can be seen coming from the gland when the ventral surface, particularly the elongated disk (Scheibe), is observed. The luminescence varies, depending on conditions of water temperature, light, etc., because of expansion or contraction of chromatophores on the ventral side of the organ. The histology is known, but no extensive physiological experiments have been carried out.

### 2. Gadidae

According to the work of Kishitani (1930) the luminous gland and the conditions for luminescence are very similar in *Physiculus japonicus* to those of *Malococephalus*. Haneda (1938, 1951) was able to see the light shining through the ventral wall but carried out no physiological experiments. Kishitani has cultured the symbiotic luminous bacteria.

### 3. Monocentridae

In the knightfish, *Monocentris japonica*, the paired light organs on the underside of the tip of the lower jaw are made up of tubules containing "granules" which Yasaki (1928) demonstrated to be luminous bacteria. Before his work, *Monocentris* had been studied by Okada (1926), who worked out the morphology and histology of the glands and made observations on the living fish, but missed the bacteria. The fish can produce light day and night but it is not continuously luminescent for 24 hours. There are periods, particularly during the day, when no light is visible. Then the light will spontaneously appear and if the water is agitated the light will continue for several hours. When ammonia is added or when in fresh water, where the fish live for 10 hours but are definitely uncomfortable, a bright light is easily observed. Yasaki has noted that light can be seen within the mouth if examined at those times when no light is showing from the outside. Okada was unable to demonstrate nerves entering the gland and the whole behavior, particularly the spontaneous luminescence, suggests chromatophore control of light intensities.

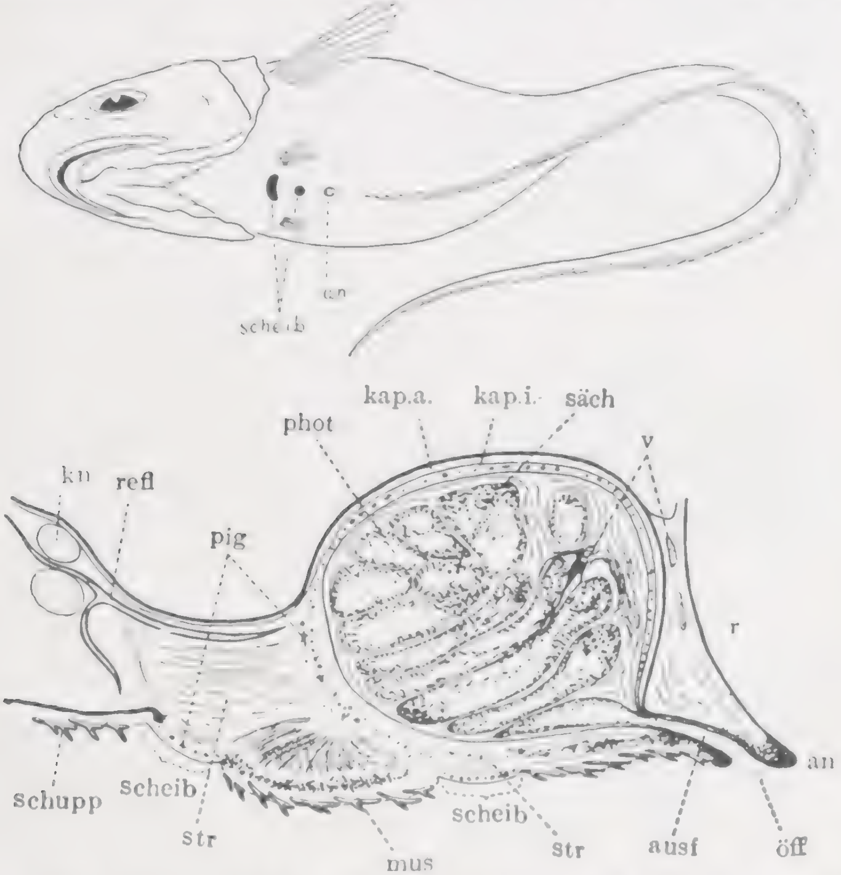

FIG. 1. Top. *Malacocephalus laevis*, showing opening of luminous gland between scheib (disk) and anus. Bottom. Section of luminous organ of the fish, showing photogenic tubules (phot), duct (ausf) and opening (öff). After Haneda.

## 4. ANOMALOPIDAE

The three genera of this family, *Photoblepharon*, *Anomalops*, and the little known *Kryptophaneron*, represent some of the most remarkable luminous fish, in that they possess a conspicuous elongated structure just under the eye, a light organ for the growth of symbiotic luminous bacteria. These organisms are found in long parallel gland tubes with abundant blood vessels passing between them (Fig. 2). At the front of the organ is a shallow reservoir with pores to the exterior and at the rear a "reflector" layer. These fish have been chiefly studied by Steche (1907, 1909), Harvey (1921, 1923) and Haneda (1943, 1953). The behavior of a suspension of gland material is exactly like that of a suspension of luminous bacteria. Bacteria are definitely present but neither Harvey nor Haneda was able to culture luminous bacteria on artificial media. Although a bacterial growth occurred it was nonluminous. Probably some essential constituent for luminescence was lacking in the culture medium.

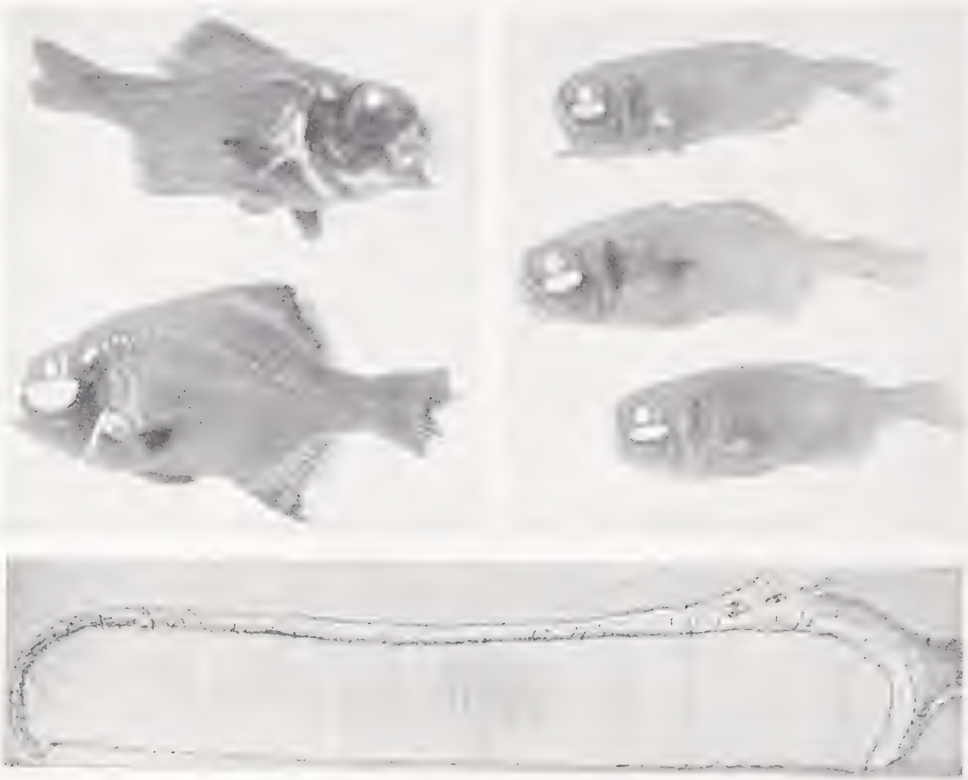

FIG. 2. Left top. *Photoblepharon palpebratus*. Right top. *Anomalops catoptron*. After Harvey. Bottom. Section of luminous organ of *Photoblepharon* showing the tubes containing luminous bacteria. After Steche.

As *Anomalops* swims through the water the light is turned on for about 10 seconds and off for about 5 seconds. *Photoblepharon* shows a light for much longer time periods (30 minutes), but as a result of excitement or of partial asphyxiation the light becomes intermittent. As the luminescence of the bacteria is continuous, control must be mechanical. In *Anomalops*, by means of the hinge at the antero-dorsal edge, the light organ can be turned downward until the light surface comes in contact with black pigmented tissue forming a sort of pocket. The light is thus cut off. In *Photoblepharon*, a fold of black tissue has been developed on the ventral edge of the organ socket, and can be drawn up over the light surface like an eyelid, thus extinguishing the light. Why these two fish, so similar in most respects, and especially in the general structure of the luminous organ, should have developed such totally different means of extinguishing the light is one of the mysteries of evolution.

Just before death from asphyxiation, when the fish swim slowly upside down, the light is usually not visible, but on actual death, when movement ceases, the organ is exposed and gives forth light, both in *Anomalops* and *Photoblepharon*. If the muscles involving the closing mechanism pass into the contracted condition on death, the relaxed condition of these muscles would appear to correspond to the closed position of the organ.

### 5. ACROPOMATIDAE

*Acropoma japonicum* emits a continuous bluish light from a large U-shaped gland in the ventral muscle tissue. There is a structure like a lens, a reflector and a long duct ending near the anus. Yasaki and Haneda (1935) and Haneda (1950) have studied the gland and grown symbiotic luminous bacteria from it.

### 6. LEIOGNATHIDAE

This family contains luminous fish in the genera *Leiognathus*, *Gazza*, and *Secutor*, with definite luminous organs of the indirect type,[1] like *Acropoma*, but consisting of a flattened ring of luminous tissue surrounding the esophagus where it joins the stomach.

The light organ is made up of finger-like glands more or less parallel to each other and radially arranged around the esophagus. Several such glands open into a short duct to a reservoir from which further ducts connect with the esophagus between the mucous villi. The contents of the ducts is made up of rod-shaped bodies which Harms (1928) working on *Equula = Leiognathus*, designated as luminous bacteria from staining reactions, although he did not attempt culture experiments. There is a sort of reflector, represented by a thick layer of tissue next to the esophagus, and a so-called lens. The light organ is well supplied with blood vessels but not nerves and in many respects resembles the light organs of the Anomalopidae. Haneda has cultured the luminous bacteria and observed the diffuse greenish glow in 15 species of Leiognathidae. The light is sometimes intermittent, with control apparently by chromatophore expansion and contraction, as it is necessary to handle the fish and remove them from the water before the luminescence is displayed.

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[1] Recently Kuwabara (1955) has described a luminous bacterial light organ of the indirect type, with a duct opening into the rectum in the fish, *Paratrachichthys prosthenius*, of the family Trachichthyidae.

### 7. Serranidae

The light of *Apogon marginatus* has been studied by Kato (1947) who wrote: "The organs are located at three distinct points of the intestine in small diverticula, one at the proximal and two at the distal end. The wall of these diverticula consists of the same type of photogenic cells, each of which is elongate in shape, striated at the free border, with a nucleus at the base and filled with an eosinophil secretion. Photogenesis appears to be intracellular. This is the first instance of an automatic luminous organ which is located in the intestinal wall." The light is presumably bacterial but the organisms have never been cultured.

### 8. Saccopharyngidae

The only observation of the light of the deep-sea eels is due to Beebe (1932) who described two troughs of whitish material along the back, scarlet pigmented papillae, and a luminous organ near the tip of the tail. He wrote: "In addition to the faint colorless glow from the nuchal troughs, we distinctly saw a faint pinkish glow and twice a flash from the specialized caudal organ, and also from several of the scarlet papillae. Under the lens I could later see blood corpuscles moving very slowly along the exposed veins."

### 9. Ceratioidea

The bacterial origin of the light from the illicium of deep-sea angler fish was suggested by Dahlgren (1928) from histological study, but no one has isolated and grown the symbiotic luminous bacteria. Light has been seen coming from the escal organ by Willemoes-Suhm in 1875, by Beebe (1926), Norman (1930), Beebe and Crane (1947), and Waterman (1939). Waterman wrote that the escal light organ at the end of the illicium of a living *Dolopichthys*, which lived in chilled sea water for 10 minutes, "remained dark until the fish was quite actively stimulated mechanically. When it did luminesce, the light was confined within the esca, which was transparent and more or less free from pigment distally, and no trace of luminous secretion was observed to be extruded from the external pore of the organ. The light was bluish green in color like that of the majority of luminous organisms and lasted five or six seconds, beginning dimly and rising to a peak in a second or so, maintaining the maximum intensity for several seconds, and then slowly fading out." This behavior suggests adrenaline or melanophore control of the luminescence. The author (1931) observed no luminescence on injecting adrenaline into a moribund *Linophryne arborifer* but further studies should be made at the first opportunity. Much of interest on these fish will be found in the great monograph of Bertelsen (1951), and in papers of Waterman (1939, 1948) and of Clarke (1950).

## B. Self-Luminous Photophores

### 1. Elasmobranchii

Since Bennett's observation in 1840, a number of the Squalidae have been described as luminous (Johann, 1899; Burckhardt, 1900; Ohshima, 1911; Hickling, 1928; Schmidt, 1930) and some observations on the stimulation of luminescence have been carried out. The light comes from many minute photophores embedded in the skin and containing photogenic cells and black pigment cells. Sometimes they are only 100 μ in diameter. All observers agree that the light appears slowly and only after considerable stimulation. In the Naples aquarium, Beer (see Johann, 1899) saw the whole underside of an injured but living *Spinax* (= *Etmopterus*) glow with a greenish light, like phosphorus, visible at 3–4 meters. It differed from phophorus in that the light spontaneously appeared and disappeared at short intervals "but invariably increased in intensity before going out. Mechanical stimulation did not affect the luminous and nonluminous regions but electrical stimulation strong enough to cause contraction of muscles released the light." When moribund, with no light visible, electrical stimulation of the spinal cord did not again excite the luminescence of Beer's specimen.

Ohshima (1911) never noted spontaneous luminescence of *Etmopterus lucifer*, but a gradual appearance and increase in light intensity over large areas when the fish was mechanically stimulated. Ammonia added to the sea water did not affect the luminescence. Since Johann could find no nerves going to the photophores of *Spinax niger* (= *E. spinax*) Ohshima concluded that changes in light intensity were connected with expansion and contraction of pigment cells acting like the iris of a camera.

Hickling (1928) noted that *Spinax niger* was not always luminous when newly caught and vigorously alive, and that it was always nonluminous when dead but the "moribund" individuals seemed most luminous. The light was rather definitely directed so that an object immediately underneath the fish and near its mouth was illuminated. "One may therefore suggest that the sudden flash of light, at the moment of attack, may cause the prey of *Spinax* to hesitate for just that fraction of a second in which the mouth can make a successful snatch."

It is obvious that many more experiments should be carried out to determine if the light organs are innervated, or if the light intensity is determined by movements of pigment cells which in turn have sympathetic innervation, and in general on the influence of adrenaline and other hormones on the process.

The only other family of elasmobranchs in which a luminous species has been reported is the Torpedinidae. Alcock (1902) caught an electric ray, *Benthobatis moresbyi*, at 430 fathoms, which "has a row of minute luminous glands along the edge of its disk, these probably being lures to attract prey." The light was not seen and nothing more is known of the animal.

## 2. STOMIATOIDEA AND MYCTOPHOIDEA

By far the greatest number of luminous species belong in these groups of bathypelagic fish (Fig. 3). Sometimes there is a luminous organ at the end of a barbel, or a moveable cheek organ as in *Astronesthes* (Haneda, 1955), similar to that of *Anomalops*, but the light mostly comes from stationary photophores arranged in a pattern on the lateral and ventral surface of the body. This pattern is constant in any one species and can be used in classification (Brauer, 1904, 1905), but male and female may have a different pattern. The histology of the various types of photophores has been thoroughly studied by many workers and the subject reviewed by Rauther (1927) and by Harvey (1952).

Of principal physiological interest is the innervation. Handrick (1901) designated the trigeminal, facial, and spinal nerves as supplying light organs in *Argyropelecus hemigymnus* and Gierse (1904) found a similar situation in *Cyclothone acclinidens*. According to Brauer (1906, 1908), the innervation is not marked but Ohshima (1911) described the photophores of *Myctophum* as richly supplied and "undoubtedly controlled by nerves." Ray (1950) also, in a careful study of the nervous system of the myctophid, *Lampanyctus leucopsarus*, found all the photophores to be innervated, those of the head by branches of the facial and those of the body by spinal nerves. Even though the light organs of the body form a linear series, they are innervated by the ventral root of the spinal nerve of the body segment in which they are located.

An early physiological study of light excitation of *Maurolicus pennantii* was made by Mangold (1907) at Naples. There was no spontaneous luminescence, nor did it appear on lifting the fish out of the sea water onto a glass plate, but strong electrical or mechanical stimulation gave a local response of those photophores near the point of stimulation, followed by others at a distance. Mangold was perplexed by this

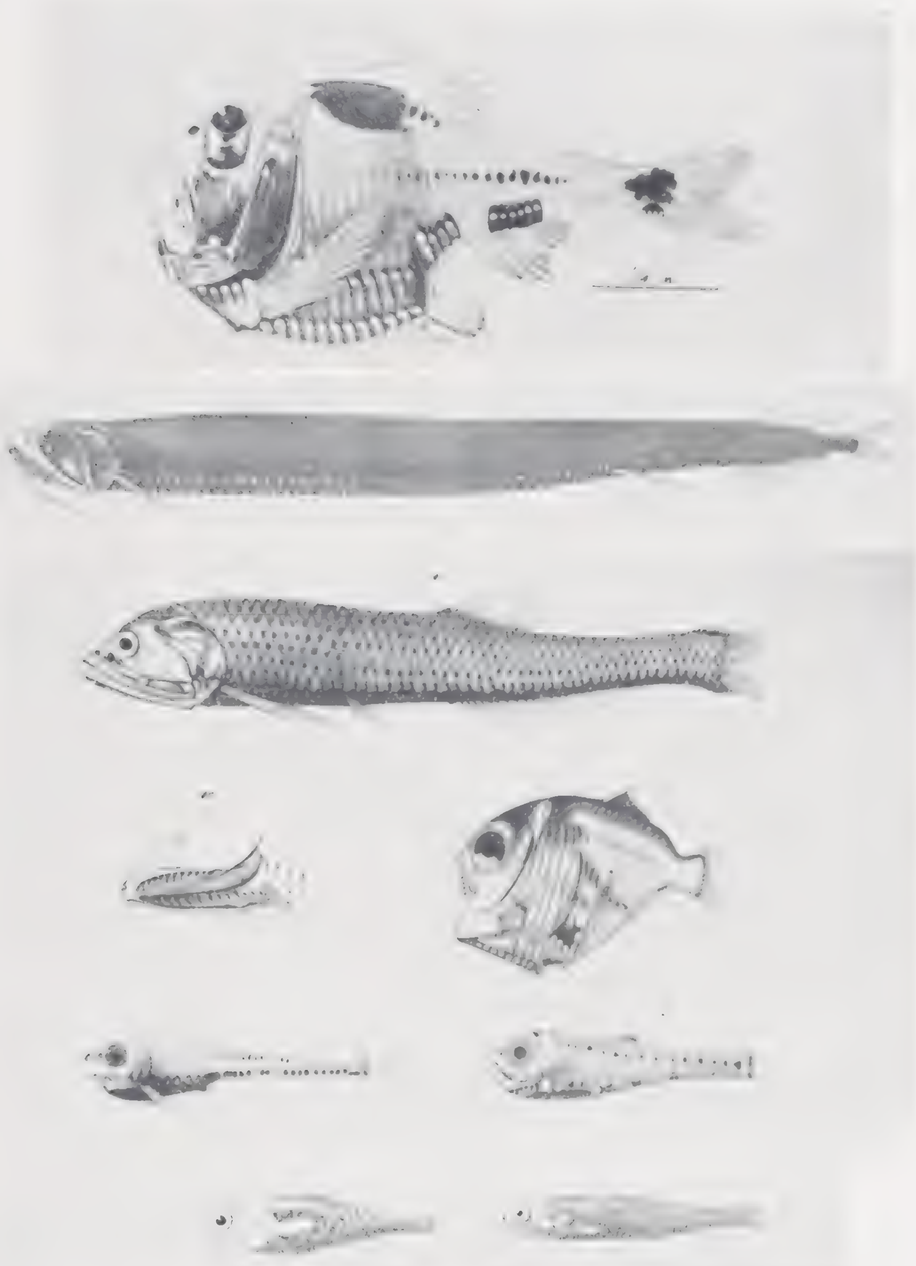

FIG. 3. Various types of deep sea fish with photophores. From the top—*Argyropelecus, Stomias, Lychnopoles, Sternoptyx, Maurolicus, Myctophum.* From various sources.

behavior, as histological study showed only sparse innervation of photophores, merely branches of skin nerves which did not look like true efferent photogenic nerves. He found that light was not excited by concentrated salt solution or by 1% pilocarpine, cocaine, or atropine in sea water, or on addition of dilute $H_2SO_4$, but immersion in fresh water caused a long lasting luminescence. The light disappeared completely on death and it was not possible to work with an excised organ, like a nerve-muscle preparation.

Ohshima (1911) made a few experiments with freshly caught *Maurolicus pennantii* on a fishing boat. Like Mangold, he noted no spontaneous luminescence, but a weak yellowish green light appeared on stimulation. Contrary to Mangold's finding, there was no light in fresh water until a few drops of formalin had been added, when a continuous feeble glow came from the photophores.

Among the Myctophidae also, (particularly *Myctophum watasei*) there was no spontaneous light, but on squeezing the fish or removing the brain, the anteorbital organs and luminous scales emitted a weak blue light, while the branchiostegals, operculars, and other scutellate organs become very weakly luminescent. The mediocaudal photophores (Leuchtplatten of Brauer) were imperfectly developed. Ohshima wrote: "By the mode of action one can distinguish two groups of the organs, the anteorbitals and the luminous scales (and probably also the mediocaudals) on the one hand, and all the other small organs on the other. The two groups act quite independently of each other, while within each all the photophores become simultaneously active. A weak stimulation was followed by the emission of light only from the second group, while the first group could be forced to action only by a stronger stimulation." There was no luminescence when the fish was placed in fresh water or dilute formalin.

Skowron (1928) has studied *Chauliodus sloanii* at Messina. These fish lived about 45 minutes in a container of sea water. They swam briskly at first, later sinking to the bottom of the vessel. In a dark room a continuous blue light could be seen along the under surface of the fish. This light was not increased on local stimulation with a glass rod, but other light organs at the point of stimulation became visible and a wave of blue light spread from the point of stimulation to both the compound (on head near eye and on sides of body) and the simple photophores. After some seconds the photophore lights went out, leaving only the weak continuous light on the belly. The stimulation could be repeated with a second lighting up of the photophores. Neither sunlight nor that of an arc lamp had any inhibiting effect on the rapidity or intensity of the luminescence.

The continuous blue light on the ventral side of the body probably comes from the cup-like organs but some bottle-like organs may also be involved, as Skowron found that the latter remained luminescent for a long time after stimulation. The continuous light on the belly disappeared when the fish sank to the bottom of the vessel and could not be revived by strong stimulation at a time when other photophores responded. When the fish died, no photophores would light on strongest mechanical stimulation, or on placing the fish in fresh water, or on treatment with ether, chloroform, or ammonia. Adding a few drops of ammonia to sea water always caused the active fish to light for some 10 seconds.

The author (Harvey, 1931) has studied 2 living individuals of *Echiostoma ctenobarba*, about 1 foot long and entirely black in color, caught at 800 fathoms south of Bermuda, and placed in iced sea water. In addition to the prominent cheek organ, *Echiostoma* has two rows of large photophores along the ventral and lateral walls as well as numerous photophores scattered over practically the whole body, including the dorsal surface. The cheek organ, pink in life, was observed to flash rhythmically with a decidedly bluish luminescence when the fish was handled, especially when it was lifted out of the sea water. No other luminescence of any kind could be noted, despite the fact that the fish was squeezed and twisted to stimulate it strongly. A hypodermic needle was then inserted but no luminescence additional to that of the cheek organ appeared. However, when 5 minims adrenaline (1:1000 in physiological saline) was injected into the side about one-third toward the tail end, there immediately appeared a yellowish luminescence of photophores near the point of injection, and soon practically all of the photophores of the fish were luminescing with a yellowish moderately intense continuous glow. This lasted a few minutes and then went out and could not be excited again by rubbing or handling but appeared as before on a second, third, and fourth injection of adrenaline. The last injection was of 10 minims and excited all organs and also the pectoral and ventral fins. There is no doubt of the luminescence of these fins despite the fact that they do not possess any easily visible light organs. No luminescence was observed in the tail, anal fins, long pectoral rays, or barbel on lower jaw. The cheek organ continued to flash at intervals after adrenaline injection but did not change in rhythm or in any noticeable way. The flashing of this organ is not due to unscreening of a continuously luminous surface. The light appears and disappears on the transparent exposed surface of organ itself. For this reason we may presume that *Echiostoma* is self-luminous and does not harbor luminous bacteria.

A most striking action of adrenaline on *Argyropelecus olfersi* has recently been described by Bertelsen and Grontved (1949). No luminescence was visible until after injection, when nearly all the photophores emitted a greenish yellow luminescence, brightest from the abdominal and lateral series. A photograph of the light was obtained (Fig. 4), which showed the bright beam due to the lens and a fan shaped portion, which came from the silvery reflector of the photophore.

It should be mentioned that adrenaline is not a stimulant for light production after a fish has been dead some time. Skowron (1928) observed no light from recently caught but dead *Chauliodus*, and the author tested several other species, even a feebly moving *Linophryne*, with negative results.

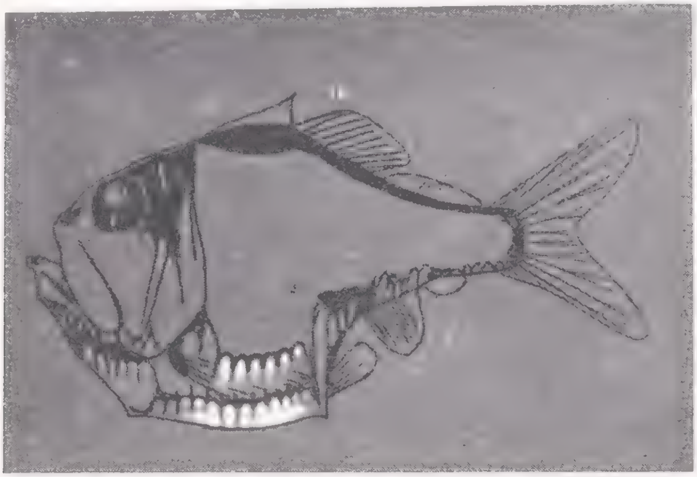

FIG. 4. *Argyropelecus olfersi,* photographed by its own light, with an outline of the fish drawn in. Photo by E. Bertelsen and J. Grontved.

Various other species, particularly *Myctophum*, have been studied by Beebe and Crane (1939) and Beebe and Van der Pyl (1944), who emphasize that individual lights or groups of lights can be flashed on independently of others, and that the lights appear to serve different purposes, the pattern of lights perhaps to identify the species and certain tail lights possibly to confuse a predator. It was observed that a *Myctophum affine* would luminesce when the luminous dial of a wrist watch was brought near.

### 3. BATRACHOIDIDAE

An extensive study of the histology, embryology and physiology of the California toadfish or singing fish, *Porichthys notatus*, was first made by Greene (1899), who found as many as 840 photophores scattered in rows over the body (Fig. 5). One hundred of the above were rudimentary and might be mistaken for lateral line organs whose pattern they often follow. These photophores are deeply embedded in the dermis, a mass of photogenic cells surrounded by a reflector and a layer of melanophores. The light passes out through a lens and a corneal-like epidermal covering. No nerves could be demonstrated by Greene but there is a rich supply of blood vessels.

Greene (1899) made many attempts to excite mechanically the luminescence of *Porichthys*, kept in aquaria, but in only one case, a fish pressed against the side of the tank, did a faint glow appear. However, if interrupted induced shocks were applied to fish on a glass plate, the line of organs on the ventral and lateral surfaces all glowed beautifully with a white light. The single well-developed organ just behind and below the eye was especially bright. No light was visible in organs that appeared rudimentary in structure. The shocks must be very strong to excite luminescence, as an intensity sufficient to cause violent contractions of muscles had no effect on the photophores. Galvanic stimulation on make and break of the current was also effective.

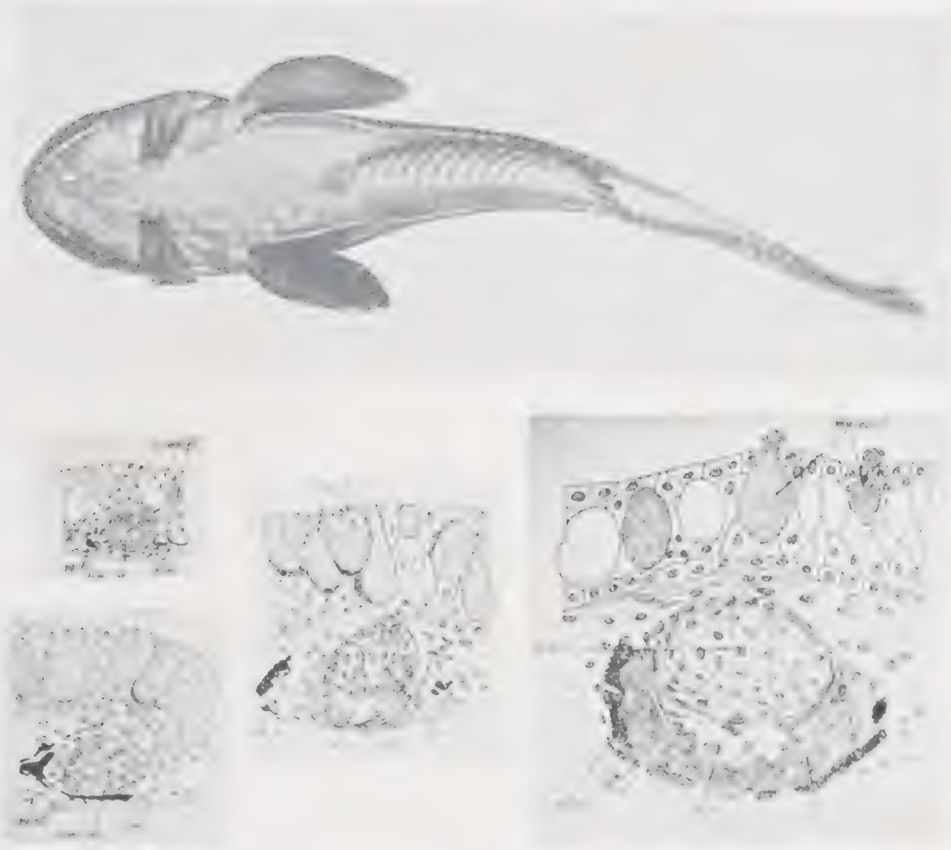

FIG. 5. Top, *Porichthys notatus*, showing the photophores. Bottom, Four stages in development of a photophore. After Greene.

The light did not appear suddenly but only after 10 seconds. It then gradually increased in intensity and gradually dimmed when the stimulation ceased. This behavior led Greene to attribute excitation by the current to a direct effect on the organ rather than an indirect one through possible nerves which he could not demonstrate histologically. The light could also be excited by addition of ammonia to the sea water. After a considerable latent period, the glow of all organs appeared, remained at a maximum for a few minutes and then over 20 minutes gradually faded out. Rubbing the skin would again call forth luminescence.

Later Greene and Greene (1924) showed that the simplest method of exciting luminescence in *Porichthys* was the injection of adrenaline. They were led to test the material because "the length of the latent period and the general character of the wave of development and waning of phosphorescence are suggestive of a chemically controlled gland rather than of one under nerve control. In order to test this hypothesis 0.25 cc. adrenaline hydrochloride was injected subcutaneously into the belly wall of the right side immediately under the prominent gastric line of phosphorescent organs. After a rather pronounced latent period, two or more spots above the area of injection began to show luminescence. These organs were not contiguous but well separated in the row. A few seconds later . . . individual organs in adjacent lines began to glow faintly, then several more organs in the posterior segment of the gastric line under which the injection occurred became visible. From this time on individual organs on both sides of the abdomen, along the anal lines and mandibular lines, that is, in regions distant from the point of injection but still in irregular areas, became visible. It was noteworthy that no true sequence or order or uniformity in the degree of light intensity occurred at first. In the course of approximately 10 minutes, the entire phosphorescent organ line system became visible and of uniform brightness." The illumination produced by adrenaline injection persisted for hours without interruption and in one experiment it was found that pituitrin also excited the luminescence.

## IV. NERVE AND HORMONE CONTROL

Light organs of some fish containing luminous bacteria are known to be controlled by nerves ending on muscles which move an opaque screen (*Photoblepharon*) or change the position of the light organ itself (*Anomalops*). In addition there are such forms as *Acropoma* and *Leiognathus*, with internal glands containing luminous bacteria, whose light is apparently increased and diminished by expansion and contraction of chromatophores in external tissues. As fish chromatophores are often controlled by sympathetic nerves, adrenergic in character (see Chap. VIII), the possibility must be considered that photophores of fish classed as self-luminous are in part regulated by chromatophore expansion and contraction and, in part, by direct nerve control of the photophore itself.

There can be no doubt of the passage of nerves to photophores of many fish and Nicol (1955) believes that these nerves are of sympathetic origin. The author's observations described above, would appear to indicate two kinds of control in *Echiostoma*, one for the cheek organ which flashed rhythmically when the fish was handled, and another for the rows of body photophores whose light was excited by injection of adrenaline.

Although Greene and Greene (1924) could not demonstrate nerve innervation in *Porichthys*, and were inclined to think that adrenaline acted as a hormone, Nicol (1955) has presented evidence that hormonal control, i.e. by circulation of hormone in the blood stream, is not essential. Electrical stimulation, which could only occur through the sympathetic system, will cause luminescence of *Porichthys*. For example, condenser shocks to the spinal cord causes photophores of both head and trunk to luminesce. The same regions respond when the circulation has been stopped by ligation of heart arteries and after transection of the cord anterior to the electrodes. In this case the only pathway to the head would be through the sympathetic chain, which is known to connect with head nerves in fish. Nicol has found that the latent period of response to electrical stimulations is 7–10 seconds, whereas it took 2 minutes before concentrated adrenaline injected into the heart could circulate and cause a general luminescence to appear.

Thus, it may well be that photophore control in fish is quite similar to melanophore control in fish. These pigment cells receive sympathetic innervation and are highly susceptible to adrenaline. It is obvious that a whole new field has been opened for the physiological study of luminescence control and it is very desirable that photocell records of the time course of a luminous response be recorded for as many different kinds of fish as possible.

## V. USE OF THE LIGHT

Although the purpose of luminescence in fish can hardly be considered a physiological subject, and definite answers are apt to be mere guesses, the extensive evolution of diverse and complicated luminous organs among both elasmobranchs and teleosts must have some meaning. The previous comparison between chromatophore and photophore systems is of special import. The chromatophore or color distribution of surface fish undoubtedly often supplies the recognition pattern by which individuals of one species recognize each other, or male recognizes female during the daytime. In the dark depths of the sea, the photophore pattern, different for each species and in some cases different in male and female, may be thought of as supplying the recognition pattern. Even if the distribution of male and female photophores is similar, it is quite possible that a different pattern of control of the lights by male and by female allows the sexes to be identified. This appears to be one important and likely use of fish photophores.

Luminescence of surface fish can be of value only at night and the above reasoning would apply to any species with nocturnal habits. It has also been suggested that light organs of fish may actually be used to illuminate the surroundings, to attract the sexes, to attract food, a lure as in the illicium of deep-sea angler fishes, a warning that the species is distasteful, or a means of defense, for confusing an attacking fish. A sudden flash of light might blind a predator, while the prey escapes. Such uses are reasonable but very difficult to prove, and the whole subject must be left for future observation and experiment.

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## CHAPTER VII

# THE PIGMENTS OF FISHES

D. L. Fox[*]

*Page*

I. Introduction . . . . . . . . . . . . . . . . . . 367
II. Flavines . . . . . . . . . . . . . . . . . . . 368
III. Pterins . . . . . . . . . . . . . . . . . . . 369
IV. Melanins . . . . . . . . . . . . . . . . . . . 370
V. Carotenoids . . . . . . . . . . . . . . . . . . 371
References . . . . . . . . . . . . . . . . . . . 383

## I. INTRODUCTION

"Our minds seem naturally inclined to devote special attention to all coloured substances" (M. Tswett). Transcending its universal esthetic appeal, pigmentation holds special attraction and significance for the biochemist and the physiologist. The colors of organic compounds are manifestations of fractional light absorption by molecular resonance, the frequency of which is retarded by chemical unsaturation. The degree of unsaturation, in turn, is a deciding factor not only of the wavelength of light selectively absorbed, but of relative chemical or biochemical reactivity; it may, for example, confer upon a compound a role as an oxidant or as a reducing agent (Fox, 1953).

Excelling the other vertebrate classes in the richness and variety of their integumentary pigmentation, the fishes thus perhaps display a sign of relatively greater diversity in their metabolism and disposition of colored molecules. Yet the compounds responsible for biochromy in fishes belong to but a few classes, namely, carotenoids, indoles (melanins), flavines, tetrapyrroles, and the pterin and purine group.

Of these classes, the yellow, orange, or red carotenoids and the brown, grey, or black melanins are by far the predominant members. The pale yellow, fluorescent flavines and the pterins are not ordinarily conspicuous, since they occur internally for the most part, or at great dilutions in some scales. The red endogenous cyclic heme tetrapyrroles, found in the blood of all vertebrates (and in the body fluids of many invertebrates), contribute in but a minor way to the external coloration of fishes; moreover, their physiological function is sufficiently well known as to demand no space for special discussion in this chapter. Their open-chained derivatives, the bilichromes, are not of very common manifestation in fishes, but impart striking green or blue-green colors to the flesh, skin, mucus, and skeleton of a few groups of fishes (Fox, 1953; Fox and Millott, 1954).

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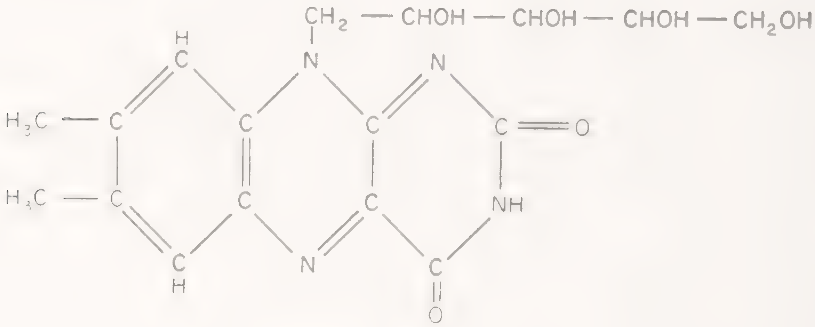
[*] (Contribution from the Scripps Institution of Oceanography, University of California; New Series No. 919.)

Finally, the purines, notably guanine, hardly belong to the true pigments, since they manifest no colors of molecular resonance. These cyclic, nitrogenous protein catabolites are mentioned here, however, because they are responsible for countless manifestations of mat whiteness (e.g., in ventral scales), of silvery iridescence or of specular blue or green colors in the skins of fishes. The interference colors of iridescence and manifestations of Tyndall blue are greatly fortified by underlying dark pigment, serving as a light screen for penetrating rays. Green aspects often arise from the combined effects of reflected blue Tyndall rays and a yellow pigment (Fox, 1953).

## II. FLAVINES

Riboflavine (benzisoalloxazine-6, 7-dimethyl-9-D-riboflavine) is crystallizable from alcohol as stellate yellow-orange needles which decompose between 274 and 293° C. The compound shows reversible optical activity in alkaline systems and, in neutral aqueous media, exhibits maximal light absorption at 445 mμ, with three ultraviolet peaks, at 372, 269, and 225 mμ, respectively.



RIBOFLAVINE

It is strongly fluorescent in pyridine, and mildly so in aqueous systems, emitting therein maximally in the green at 562–565 mμ. This property shows little variation between pH 3 and 9, and is nearly constant between pH 5.5 and 8.0. Conjugated as flavoproteins, the natural state *in vivo*, these compounds are not fluorescent, and possess molecular weights of from 60,000 to 77,000.

Concentrations of flavines, amounting to but a few micrograms per gram, show progressive decreases in the eggs of several elasmobranch species after fertilization, and in the developing embryonic shark, Ce-

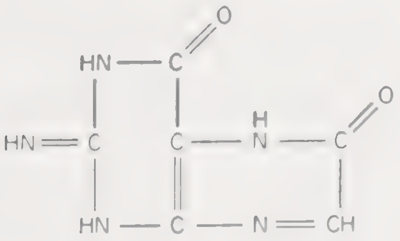
*charhinus commersonii* (Fontaine and Gourevitch, 1936).

But developing free-swimming stages of the eel, *Anguilla anguilla*, show increasing concentrations of flavines, reaching a peak in the adult male, wherein the dorsal and flank skin (but not the white ventral) carries the richest supplies, closely associated with maximal concentrations of carotenoids and melanins, and with minimal or no guanine-containing leucophores (Fontaine, 1937). The correlation of rich flavine deposits with localization of epidermal melanophores holds not only for the eel but also for the scales of the carp and in the conger, *Conger conger*, the blennies, *Blennius gattorugine* and *Muraena helena*, and the lamprey, *Petromyzon marinus* (Fontaine and Busnel, 1938).

Riboflavine has been found to catalyze, *in vitro*, the photochemical oxidation of some pterins, aromatic amino acids, and purines, while aliphatic amino acids are hardly affected (Isaka and Kato, 1952). Cupric, ferric, and to lesser degrees, cobaltous, manganous, nickelous, and zinc ions inhibit this oxidative power of photoactivated riboflavine, while magnesium and calcium ions are ineffective. The inhibitory divalent cations lead to decreases in fluorescence intensity, presumably through chelation between the metal and the photoriboflavine (Isaka and Ishida, 1953).

## III. PTERINS

Pterins, involving 30–40% organically combined nitrogen, are somewhat amphoteric, showing limited solubility in mild acids or alkalis, less in neutral aqueous media, and insolubility in common organic solvents. Some of them give a positive murexide reaction, like the purines. The pterins tend to decompose rather than to melt, hence must be diagnosed by their relative nitrogen content, their absorption spectra, fluorescent properties, and behavior toward adsorptive substances (Fox, 1953, and cited references).



Xanthopterin

Like the flavines, pterins occur in very minute concentrations, in various tissues of fishes and other animals, but possess certain important physiological attributes. Simmons and Norris (1941) and Norris and Simmons (1945) determined that xanthopterin, a yellow member of the series, injected in doses of but 30–50 μg., restored juvenile salmon (several *Oncorhynchus* species) from nutritional anemia.

A blue-fluorescing pterin, fluorescyanin (ichthyopterin, $C_7H_7O_3N_5$), encountered by Fontaine and Busnel (1939) in fishes' scales, is discussed extensively by Polonovski, Busnel, and Pesson (1946). Like riboflavine, fluorescyanin occurs as a chromoprotein and, when free, is reduced by dithionate to a non-fluorescing leuco form, but less rapidly so than riboflavine. Its more ready reoxidation by atmospheric oxygen has led to the hypothesis that it may serve as a hydrogen transporter. The more richly pigmented dorsal scales of carp or goldfish yield more fluorescyanin than do the pale ventral scales, and consume about $\frac{1}{3}$ more oxygen per hour. Physiological experiments have indicated a similarity between fluorescyanin (and certain other pterins) and $B_1$ and $B_2$ vitamins (Polonovski *et al.*, 1946).

Some Japanese workers have studied the possible photochemical role of some pterins. Yagi (1954) reports the inhibition of the photodecomposition of riboflavine by pterins (in frog skin) but not through any primary role as light-absorbing screens. Kushibiki, Hama, and Gota (1954) describe some violet-fluorescing pterins, cyprinopurple $A_1$ and $A_2$ (very similar to fluorescyanin) and cyprinopurple B (a photochemical derivative of $A_2$) from the scales of carp, *Cyprinus carpio* and *Carassius auratus*. The three pterins, separated by paper chromatography, are all reversibly reducible by dithionate, and exhibit characteristic changes in fluorescence with varying pH and on ultraviolet irradiation.

The consumption of oxygen by dopa is accelerated by xanthopterin, the more so if in the presence of cupric ions, while leucopterin is ineffective, whether alone or with copper. This was found by Isaka and Shuzo (1953), who also confirmed the role of zinc ions in catalyzing the oxidation of dopa-chrome (red) to the colorless phase, regarded as the second step in melanogenesis. Addition of xanthopterin to the system weakens the effect of the zinc, perhaps by chelative binding, or perhaps, according to Isaka (1952), by inhibiting the oxidative reaction, i.e. by competing with dopa for the available oxygen, giving rise to phenylalanine quinone, leucopterin, and water.

## IV. MELANINS

The dark, endogenous melanins, which are oxidized, polymerized end-products from the metabolism of tyrosine and allied phenolic compounds, are well known for their black pigmentation of eyes, peritoneal lining, and exposed areas of skin. Mobile melanin granules within the dendritic chromatophores of fishes' scales are responsible for the striking reversible chromatic changes (Sumner, 1940; Parker, 1948; and see Chap. VIII). Melanin aggregates in deeper layers of skin, beneath pigment-free, turbid overlayers of tissue, give rise to the familiar Tyndall blue colors.

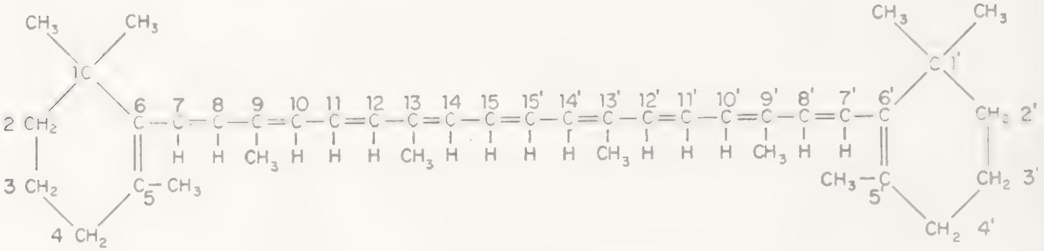
Many ceratioid fishes display intensely black pigmentation over the entire body surface, e.g. the black sea devil *Melanocetus johnsoni*, a deep-sea angler fish, has a naked, slimy skin of shiny, jet-black color, save for the small eyes and the conical luminescent tip of the esca or whip-like lure extending from the snout.

At least one species of crestfish (*Lophotus*) stores copious melanin pigment in a specialized glandular sac, whence it is expelled forcibly through a post-anal aperture by muscular contractions against the bifurcated air-bladder lying against either side of the gland. The viscid, adhesive ink has a musty or seminal odor, and, on extraction with ether–acetone solution, yields a little yellow sterol-containing lipid material (cf. octopus ink, Fox and Crane, 1942). The melanin, believed to be conjugated with protein, can be dissociated therefrom by alkaline hydrolysis, and is thereafter readily reprecipitable by mild acidification. Also, like other melanins, the black or sepia-colored centrifugate in this instance was insoluble in ether, alcohol, or acetone, but dissolved in pyridine and in ethylene chlorhydrin. It was readily bleached by hydrogen peroxide (Fox and Hubbs, unpublished).

Pronounced melanogenesis has long been recognized in association with pathological conditions and with moribundity in fishes. Gordon (1948) has reviewed the genetically linked development of melanotic tumors from aggregations of aberrant macromelanophores in several species of fishes (see Chap. X). And Blackburn (1950) discusses the rapid integumentary blackening of the Tasmanian whitebait *Lovettia seali* in the post-spawning, moribund condition. The melanophores increase conspicuously in numbers with successive stages of sexual maturity, especially in the males, and spawned individuals exhibit large dark areas or, notably when dying, complete blackening of the skin. These observations emphasize the association between degradation of tyrosine into melanin, and accelerated protein metabolism, whether concerned with the rapid proliferation of cells, either gonadal or neoplastic, or with cellular decomposition.

## V. CAROTENOIDS

Because the carotenoids show considerable contrast with the other biochromes of fishes, in respect to their extensive variety within the chemical class, their relatively high local concentrations, and their visible and metabolic conspicuousness, they have been assigned most of the limited space allotted to this chapter. They are lipoid-soluble, chemically unsaturated, nitrogen-free compounds of high molecular weight (ca. 536), involving only carbon and hydrogen (carotenes; $C_{40}H_{56}$), or these elements plus oxygen (xanthophylls or esters thereof). The molecular configuration responsible for color manifestation is a so-called conjugated double-bond system, wherein the unsaturated linkages occur between alternative pairs of carbon atoms in a long, multiply branched chain. Most, but not all of the known carotenoids have an ionone-like trimethyl substituted cyclohexenyl ring at each end of an 18-carbon chain.



β-carotene

At certain C atoms, one H is replaced by oxygen-containing polar groups, e.g. by –OH at C–3 or C–3′ in cryptoxanthin, at each of these loci in zeaxanthin and in astaxanthin, which has also enolizable (thus acidogenic) keto O at both C–4 and C–4′, while its artifact, astacene, is 3,3′,4,4′-tetraketo-β-carotene. α-Carotene and its hydroxy derivatives contain the double bond between C–4′ and C–5′, thus involving less symmetry. Absence of the bond between C–1 and C–6 characterizes γ-carotene, encountered in the plant dodder and in some fungi, while a completely open chain (by absence of the opposite link between C–1′ and C–6′ as well) represents lycopene, the red pigment of tomatoes and watermelons. There is a very large family of oxygen-containing carotenoids, the formulae for which are depicted in several standard treatments of the subject (e.g. Karrer, Jucker, and Braude, 1950).

Carotenoids occur in fishes, as in most animals, chiefly dissolved in fatty droplets present in eggs, gonads, liver, and skin, to which latter they often confer strikingly conspicuous yellow, orange, or red colors. The xanthophylls, or oxygen-containing carotenoids, often as esters, predominate greatly over the carotenes or hydrocarbon members, both in incidence and in relative concentrations. Indeed, many species of fishes apparently store solely xanthophylls, rejecting or destroying dietary carotenes completely.

Methods for the recovery and resolution of carotenoids into their various fractions are described in other publications (e.g., Fox 1953 and cited references). Diagnostic characteristics of this general class of biochromes may be summarized as follows:

(1) Color in solution red, orange, or yellow, depending on molecular polarity, concentration, and solvent.

(2) Soluble in various organic fat solvents; insoluble in water.

(3) Blue or green colors on treatment of a chloroformic solution with concentrated sulfuric acid or with a solution of antimony trichloride in chloroform.

(4) Distribution between petroleum ether and 90 to 96% methanol (the so-called partition or phase test):

- (a) Carotenes persistently epiphasic (remaining in petroleum ether).
- (b) Free xanthophylls hypophasic (migrating to the aqueous methanol layer).
- (c) Esters of xanthophylls or of acidogenic carotenoids epiphasic.
- (d) Following saponification and dilution, carotenoid acid soaps collect as colored layer at interface.

(5) Chromatographic adsorption:

- (a) Xanthophylls selectively adsorbed from petroleum ether upon powdered calcium carbonate.
- (b) Carotenes, unadsorbed by calcium carbonate, adsorbed upon powdered magnesia, alumina, or calcium hydroxide.

(6) Absorption spectra:

- (a) Solutions of carotenes and of most xanthophylls manifest two absorption bands, in the blue-green, blue or blue-violet, sometimes with a faint third shadow in the violet region of the spectrum.
- (b) Solutions of carotenoid acids, and of certain unusual neutral xanthophylls, show a single broad absorption band in the blue region.

The body of evidence accumulated to date indicates that fishes belong to a class of metabolic selectors of conventional xanthophylls, rejecting all or most of the dietary carotenes. A comparative biochemical survey has yielded little, to date, of an evolutionary trend. Table I presents a review of the occurrence of carotenoids in numerous species of fishes (see Goodwin, 1952; Fox, 1953), listed in successive order of ascending families.

Carotenes (largely $\beta$-carotene) seem to occur but rarely in fishes, in very small concentrations, and then usually in glandular structures such as liver and ovaries or eggs. Traces have been reported in the flesh of certain salmons and in the skin of only two or three of the species listed, namely the burbot, *Lota lota*, the river perch, *Perca fluviatilis*, and possibly the goldfish, *Carassius auratus*. Zeaxanthin-like carotenoids are likewise scarce in fishes, having been reported in the eggs of the trout, *Salmo irideus,* in the skin of *Perca fluviatilis*, and questionably also in that of the two species of topminnow, *Xiphophorus*, the goldfish, *Carassius auratus*, and the gourami, *Macropodus*. Astaxanthin-like carotenoids were detected in about a third of the fourscore genera listed. Of these, some 18 instances referred to skin, flesh, or eyes, and 9 to liver, ovaries, or eggs. There are no conspicuous differences between the incidence of astaxanthin among the soft-rayed or intermediate classes of teleosts on the one hand, and the spiny-rayed group on the other.

The predominant integumentary carotenoids of fishes belong chiefly to the taraxanthin and lutein classes. No outstanding differences in the distribution of these two xanthophylls are yet apparent, and there is need for a more extensive survey. Neglecting the two cyclostome species and the pair of elasmobranchs at the lower end of the evolutionary scale, each of whose skin yielded lutein, the skin of soft-rayed and intermediate teleost species seem to be about equally distributed as to incidence of lutein and of taraxanthin, while a slight preponderance of taraxanthin is apparent in the spiny-rayed group. Of 40 species in the first group, 10 (25%) contain only lutein, while 9 contain taraxanthin alone, and another 9 (22% each) yield both types. In the skins of 40 spiny-rayed species, there are 13 (32%) carrying taraxanthin alone, only 7 (17%) lutein alone, and 12 (30%) yielding both lutein and taraxanthin (or violaxanthin). It should be pointed out that the terms "lutein," "taraxanthin," "violaxanthin," and the like, as used here, are not sharply definitive, but commonly refer to xanthophyllic carotenoids which are undifferentiable from those respective compounds in spectral and other properties, save perhaps by mixed chromatography.

The food and feeding habits are insufficiently different to lead to any correlations with type of carotenoid stored, since the great majority of fishes are carnivorous, and a fair number omnivorous. There appear to be somewhat more "lutein selectors" and fewer "taraxanthin selectors" among the freshwater species, most of which belong to the lower or to the intermediate evolutionary group.

Fishes seem, despite the wide occurrence and conspicuousness of their red, orange, and yellow pigmentation, to store but few unusual carotenoids, adhering chiefly to xanthophylls of the taraxanthin, lutein, and astaxanthin types, probably in that order of frequency. The brightly colored opah, *Lampris regius*, is exceptional, storing in its lips, tongue, and fins a striking red esterified xanthophyll, neutral after hydrolysis but showing a single broad symmetrical absorption band with a maximum at 472 mμ in petroleum ether, and at 502–504 mμ in carbon disulfide, thus close to the respective maxima of astacene. The opah's heavy diet of nautiloid cephalopods suggests that its pigment may have an ultimate crustacean origin, but there remains the question as to how astaxanthin may be converted by the fish, or by the molluscan intermediary, into a nonacidogenic carotenoid (Goodwin and Fox, 1955).

Less surprising than the rejection of carotenes by most fishes is the finding, in one species at least (*Fundulus parvipinnis*) of an apparent ability to convert dietary β-carotene into the familiar taraxanthin class of pigment. Moreover, another taraxanthin-selecting species, the bright orange marine goldfish *Hypsypops rubicunda*, ultimately stores small amounts of carotene in its skin after having been long maintained upon a xanthophyll-free, carotene-rich diet (see below).

Relatively little quantitative work has been done on carotenoid metabolism in fishes, but brief summaries of such experiments should be presented here. Both immature sexes of the Pacific killifish, *Fundulus parvipinnis*, yield approximately 1 mg. of taraxanthin-like xanthophyll per 100 g. of fresh tissues. Sexually mature males store as much as 36% more of the pigment in their skins than do the females, which mobilize relatively large amounts of the unesterified xanthophyll into the eggs. Despite pronounced associated chromatophoric color changes in the direction of matching and concealment, immature *Fundulus* yielded little or no variations in xanthophyll content after protracted residence (1–2 months) in black, white, red, or yellow containers. Nor did 6 weeks in total darkness elicit any measurable change in this regard.

Not only did growing *Fundulus* suffer no losses in total quantities of integumentary xanthophylls after 14 weeks on a carotenoid-deficient diet, but gains occurred in both concentration and total quantities of taraxanthin when a diet rich in that pigment was maintained over the same interval. Moreover, in a third lot of specimens fed no xanthophylls but only β-carotene, significant increases in the quantities of taraxanthin stored led to the conclusion that the fish were able to effect conversion of a carotene into a xanthophyll (Sumner and Fox, 1933, 1935a).

The brilliant omnivorous marine goldfish or Garibaldi, *Hypsypops rubicunda*, bears rich supplies of taraxanthin-like esters in its skin, yielding from about 24 to 98 mg. per 100 g. of skin according to age. The stores of integumentary xanthophyll remain unchanged for long periods, decreasing but slowly after months on carotenoid-free diets. Individuals captive for 2 to 3 years lost from 81 to 88% of their skin carotenoids. Gradual losses in xanthophyllic pigment were found even after rich supplies of β- and α-carotene had been included in the food

TABLE I

DISTRIBUTION OF CAROTENOIDS THROUGH THE ASCENDING GROUPS OF FISHES

| Class, Order, Family | Genera, Species (food habit: C = carnivores D = detritus feeders O = omnivores H = herbivores P = plankton eaters) | Tissues | Carotenoid Types | | | | |
|---|---|---|---|---|---|---|---|
| | | | Tara-xanthin | Lutein | Zea-xanthin | Asta-xanthin | Caro-tene |
| CYCLOSTOMES | | | | | | | |
| Petromyzontidae (lampreys) | *Lampetra fluviatilis* | | | | | | |
| | larva (D) | Whole larvae | | + | | | |
| | adult (C; parasitic) | Adult skin | | + | | | |
| | *Lampetra planeri* | | | | | | |
| | larva (D) | Whole larvae | | + | | | |
| | adult (does not feed) | Adult skin | | + | | | |
| ELASMOBRANCHS | | | | | | | |
| Rajidae (skates) | *Raja batis* (C) | Skin | | + | | | |
| | *Raja clavata* (C) | Skin | | + | | | |
| TELEOSTS | | | | | | | |
| A. Soft-rayed fishes | | | | | | | |
| Clupeidae (herrings, sardines) | *Clupea harengus* (P) | Skin | + | + | | | |
| Anguillidae (eels) | *Anguilla anguilla* (C) | Skin | | + | | | |
| Coregonidae (whitefishes) | *Coregonus albula* (P) | Flesh | | | | + | |
| | | Ovaries, eggs | | | | + | |
| | | Skin | | | | + | |
| Salmonidae (salmons, trouts) | *Salmo gairdneri* (C) | Flesh, skin | | | | + | |
| | *Salmo irideus* (C) | Flesh | | | | + | |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | | Eggs | | + | + | + | + |
| | | Skin | | + | | + | |
| | *Salmo salar* (C) | Flesh | | | | + | (+) |
| | | Eggs | + | | | | |
| | | Liver | | + | | | |
| | | Skin, fins | | + | | + | |
| | *Salmo trutta* (C) | Flesh (red) | | + | | + | (+) |
| | | Eggs | + | + | | | + |
| | | Liver | | + | | | + |
| | | Skin, fins | + | + | | + | |
| | | Eyes | (xanthophyll traces) | | | | |
| | *Salvelinus* spp. (C) | Skin | | | | + | |
| | *Oncorhynchus nerka* (C) | Flesh | | | | + | |
| | | Skin | | | | + | |
| Esocidae (pikes) | *Esox lucius* (C) | Skin | + | | | | |
| Cyprinidae (minnows) | *Barbus nigrofasciatus* (C) | Skin | | | | + | |
| | *Carassius auratus* (O) | Skin | + | + | +? | + | (+) |
| | *Leuciscus* | | | | | | |
| | (= *Rutilus*) *rutilus* (O) | Liver | | + | | + | |
| Erythrinidae (characins) | *Copeina guttata* (C) | Skin | + | | | | |
| Cyprinodontidae (killifish) | *Fundulus parvipinnis* (O) | Skin, viscera | + | | | | |
| | *Oryzias latipes* (C) | Skin | | + | | | |
| Poeciliidae (topminnows) | *Platypoecilus* (= *Xiphophorus*) | | | | | | |
| | *maculatus* (O) | Skin | | + | +? | | |
| | *Xiphophorus helleri* (O) | Skin | | + | +? | | |
| B. Intermediate Groups | | | | | | | |
| Anacanthines | | | | | | | |
| Gadidae (cods) | *Eleginus navaga* (C) | Eggs, ovaries | (three unidentified xanthophylls) | | | | |
| | *Gadus* (= *Melanogrammus*) | | | | | | |
| | *aeglefinus* (C) | Skin | + | | | | |
| | *Gadus callarias* | | | | | | |
| | (= *Gadus morrhua*, see below) | | | | | | |

TABLE I (Continued)

Distribution of Carotenoids through the Ascending Groups of Fishes

| Class, Order, Family | Genera, Species | Tissues | Carotenoid Types | | | | |
|---|---|---|---|---|---|---|---|
| | | | Tara-xanthin | Lutein | Zea-xanthin | Asta-xanthin | Caro-tene |
| | *Gadus* (= *Odontogadus*) *merlangus* (C) | Skin | + | | | | |
| | *Gadus* (= *Trisopterus*) *minutus* (C) | Skin | + | + | | | |
| | *Gadus morrhua* (C) | Skin | + | + | | | |
| | | Eggs | | + | | + | + |
| | *Gadus* (= *Pollachius*) *pollachius* (C) | Skin | + | | | | |
| | *Gadus* (= *Pollachius*) *virens* (C) | Skin | + | + | | | |
| | *Gaidropsarus* (= *Enchelyopus*) *cimbrius* (C) | Skin | | + | | | |
| | *Gaidropsarus mustelus* (= *Ciliata mustela*) (C) | Skin | | + | | | |
| | *Lota lota* (C) | Skin | + | | | | + |
| | | Eggs | | + | | | + |
| | *Raniceps raninus* (C) | Skin | | + | | | |
| Allotriognaths | | | | | | | |
| Lampridae (opahs) | *Lampris regius* (C) | Skin | | | | + (nonacidogenic ester) | |
| Regalecidae (oarfish) | *Regalecus glesne* (C) | Liver | | | | + | |
| Flatfishes | | | | | | | |
| Scophthalmidae (= Bothidae) | *Bothus maximus* (= *Psetta maxima*) (C) (= *Scophthalmus maximus*) | Skin | + | | | | |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | *Bothus* (= *Scophthalmus*) *rhombus* (C) | Skin | + | + | | | |
| | *Scophthalmus* (= *Phrynorhombus*) *norvegicus* (C) | Skin | | | | + | |
| Pleuronectidae | *Hippoglossus hippoglossus* (C) | Eggs, ovaries | | + | | + | + |
| | *Pleuronectes* (= *Platichthys*) *flesus* (C) | Skin | | + | | | |
| | *Pleuronectes* (= *Microstomus*) *kitt* (C) | Skin | + | + | | | |
| | *Pleuronectes* (= *Limanda*) *limanda* (C) | Skin | + | + | | | |
| Soleidae | *Solea vulgaris* (= *Solea solea*) (O) (D) | Eggs, ovaries | | | | | + |
| C. Spiny-rayed Fishes | | | | | | | |
| Berycoids | | | | | | | |
| Berycidae | *Beryx decadactylus* (C) | Skin | | | | + | |
| Bass-like fishes | | | | | | | |
| Serranidae (sea basses) | *Centropristes striatus* (C) | Eyes | + | | | | |
| Percidae (perches) | *Perca fluviatilis* (C) | Skin | + | | + | + | + |
| Carangidae (jacks, yellowtails) | *Caranx trachurus* (C) | Skin | | + | | | |
| Scombridae (mackerels) | *Scomber scombrus* (C) | Skin | + | + | | | |
| Sparidae (porgies) | *Stenotomus chrysops* (= *versicolor*) (C) | Eyes | + | | | | |
| Embiotocidae (viviparous perches) | *Cymatogaster aggregata* (C) | Skin, gut | + | | | | |
| Pomacentridae (damselfishes) | *Hypsypops rubicunda* (C, H) | Skin | + | | | | |
| Labridae (wrasses) | *Centrolabrus exoletus* (C) | Skin | + | + | | + | |
| | *Crenilabrus melops* (C) | Skin | + or | + | | | |

TABLE I (Continued)

Distribution of Carotenoids through the Ascending Groups of Fishes

| Class, Order, Family | Genera, Species | Tissues | Carotenoid Types | | | | |
|---|---|---|---|---|---|---|---|
| | | | Tara-xanthin | Lutein | Zea-xanthin | Asta-xanthin | Caro-tene |
| | *Ctenolabrus suillus* (C) | Skin | + or | + | | | |
| | *Labrus bergylta* (C) | Skin | | + | | | |
| | *Labrus ossifagus* (C) | Skin | | + | | | |
| Girellidae (opaleyes) | *Girella nigricans* (O) | Skin | + | | | | |
| Scorpaenoids | | | | | | | |
| Scorpaenidae (scorpionfishes) | *Scorpaena scrofa* (C) | Skin | | | | +? | |
| | *Sebastes marinus* (C) | Skin | | | | + | |
| Cottidae (sculpins) | *Cottus bubalis* (C) | Skin | + | + | | | |
| Triglidae (gurnards) | *Trigla gurnardus* (C) | Skin | + | | | | |
| | *Prionotus carolinus* (C) | Eyes | | | | + | |
| Cyclopteridae (lumpfishes) | *Cyclopterus lumpus* (C) | Skin | | | | + | |
| | | Liver | | | | + | |
| Gobies | | | | | | | |
| Gobiidae (gobies) | *Aphia minuta* (C) | Skin | + | | | | |
| | *Gillichthys mirabilis* (C) | Skin | + | | | | |
| | *Gobius niger* (C) | Skin | | + | | | |
| Labyrinthfishes | | | | | | | |
| Anabantidae (gouramis) | *Betta splendens* (C) | Skin | | + | (also violaxanthin?) | | |
| | *Colisa fasciata* (C) | Skin | | + | (also violaxanthin?) | | |
| | *Colisa lalia* (C) | Skin | | + | (also violaxanthin?) | | |
| | *Macropodus opercularis* (C) | Skin | | + | +? | | |
| Trachiniforms | | | | | | | |
| Trachinidae (weevers) | *Trachinus draco* (C) | Skin | + | | | | |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Callionymidae (dragonets) | *Callionymus lyra* (C) | Skin | + | | | | |
| Blennioids | | | | | | | |
| Pholidae (gunnels) | *Pholis gunnellus* (C) | Skin | + | + | | | |
| Zoarcidae (eel-pouts) | *Zoarces viviparus* (C) | Skin | + | + | | | |
| Ammodytidae (sand-lances) | *Ammodytes* (= *Hyperoplus*) *lanceolatus* (C) | Skin | + | | | | |
| | *Ammodytes tobianus* (C) | Skin | + | | | | |
| | | Ovaries, eggs | + | | | | + |
| Syngnathiforms | | | | | | | |
| Gasterosteidae (sticklebacks) | *Gasterosteus aculeatus* (C) | Skin | + | | | | |
| Syngnathidae (pipefishes, sea horses) | *Nerophis* (= *Entelurus*) *acquoreus* (P) | Skin | | + | | | |
| | *Nerophis ophidion* (P) | Skin | + | | | | |
| | *Siphostoma typhle* (P) | | + | + | | | |
| | *Syngnathus acus* (P) | | + | + | | | |
| Pediculates | | | | | | | |
| Lophiidae (anglers) | *Lophius piscatorius* (C) | Liver | + | + | | — | —? |
| Plectognaths | | | | | | | |
| Molidae (molas) | *Orthagoriscus* (= *Mola*) *mola* (C) | Liver | | | | — | —? |

although it was ultimately determined that traces of carotene had been assimilated and stored as such after a long period on the specialized diet (Kritzler *et al.,* 1950).

The remarkably euryhaline long-jawed goby, *Gillichthys mirabilis,* likewise possesses integumentary taraxanthin, in concentrations about half those of *Fundulus.* Like *Fundulus, Gillichthys* demonstrated no recognizable quantitative changes in xanthophyll following protracted residence on the various colored backgrounds to which they adapted their body color. Again, like *Fundulus,* this goby may be capable of converting dietary carotene into integumentary xanthophyll (Sumner and Fox, 1933). Mr. Arthur Lockley, working in the writer's laboratory, has verified this, and has found a loss of skin xanthophyll in gobies which had been required to adapt themselves osmotically to concentrations of sea salts above normal. Decreases in skin xanthophyll were associated with increased respiratory activities, suggesting that work may have been performed by the skin in order to maintain an osmotic equilibrium.

Immature greenfish or opal-eye, *Girella nigricans,* unlike *Fundulus* and *Gillichthys,* lose their integumentary taraxanthin in captivity, despite the inclusion of such carotenoids in their diet. Moreover, the rate of loss is more rapid when the fishes are kept on white backgrounds than when on yellow or red, and least rapid on black (Sumner and Fox, 1935b).

The yellow-banded viviparous surf perch, *Cymatogaster aggregata,* feeding upon shrimp (e.g. *Hippolyte californiensis*), rejects therefrom the carotenes, as well as an astaxanthin-like, acidogenic carotenoid which is, nevertheless, first hydrolyzed from its esterified condition in the gut. A taraxanthin-like xanthophyll ester from the shrimp was also hydrolyzed, then absorbed in part and stored, in re-esterified form, in the skin. Any quantities which may exceed the consumer's apparent threshold of assimilation (ca. 0.27 mg. per 100 g. of wet, eviscerated fish) remain uncombined and are stored in the rectal segment of the gut, temporarily conferring upon it a yellow, rich orange, or reddish color. When the critical xanthophyll is withheld from the diet, the rectal segment readily loses its color, while the appearance of the skin and the quantity of xanthophyll extractable therefrom remain unchanged even after some weeks( Young and Fox, 1936).

We owe to Steven (1948, 1949) a better understanding of the kinds, disposition, and metabolism of carotenoids in the brown trout, *Salmo trutta.* Carotene is present in liver and ovaries, lutein and astaxanthin in skin, muscles, and ovaries, lutein alone in eyes, and predominantly in the liver. The integumentary xanthophylls are esterified, as in many if not most other fishes; the optical lutein is also esterified, while the xanthophylls of muscles and ovaries are uncombined, and those of the liver partly esterified, partly free. Direct comparative microspectrophotometric inspection led Steven (1948) to conclude that the yellow xanthophores carry only lutein esters (1 to 3 × 10[illegible] γ per cell), and the red erythrophores some 2 to 3.5 × 10[illegible] γ of astaxanthin per cell. Trout deprived of dietary carotenoids lost most of their red and yellow pigmentation, which could be restored by feeding a natural supply of the appropriate source of pigment. Grangaud *et al.* (1952) conducted similar feeding experiments on other salmon species with like results. *Salmo trutta* is, like most fishes, apparently unable to convert one type of carotenoid into another.

Mature female trout seem to lose free lutein and astaxanthin from their muscles to the developing ova, while the esterified skin xanthophylls remain undepleted. The free yolk xanthophylls are absorbed by the embryo, re-esterified, and ultimately deposited chiefly in skin and fins. β-Carotene, detectable in freshly deposited eggs, was not recognized in any of the developmental embryonic stages. Microsurgical removal of 90% of the yolk carotenoids resulted in the larvae appearing normal in all respects save for slight reduction in size and bearing none save a few pale chromatophores (Steven, 1949).

The metabolism of astaxanthin in the fertilized egg of *Salmo salar* has been followed by Glover, Morton, and Rosen (1952) who found it to be the sole carotenoid present; not even any A vitamins or 7-dehydrocholesterol were detectable during the first 3 months of development. Most of the astaxanthin is assimilated by the developing embryo, and there stored in esterified form.

One may hardly survey the extensive occurrence of carotenoids in the skin and ovaries of fishes, and indeed in comparable tissues of countless invertebrates as well, without reflecting on the possibility of a biochemical role played therein by these biochromes, comparable to functions fulfilled by the related A vitamins in other animals.

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## Chapter VIII

# COLOR CHANGES

J. M. Odiorne

Page
I. Introduction . . . 387
II. Physiological Color Changes . . . 388
  A. Chromatophoral Responses . . . 388
    1. Pigmentary Migration . . . 388
      a. Nature . . . 388
      b. Time required . . . 389
      c. Pulsation . . . 389
      d. Rest and activity . . . 389
      e. Fatigue . . . 390
    2. Physical Color Changes . . . 390
  B. Modes of Control . . . 391
    1. Hormonal Control . . . 391
      a. Pituitary hormones . . . 391
      b. Adrenaline . . . 393
      c. Other hormones . . . 393
    2. Neural Control . . . 393
      a. The neurohumoral theory . . . 394
      b. Nature of chromatophoral nerve fibers . . . 395
    3. Receptors . . . 395
      a. The eyes . . . 395
      b. Central nervous organs . . . 396
      c. The skin . . . 396
    4. Comparative Account of Chromatophoral Control . . . 396
      a. Cyclostomata . . . 396
      b. Elasmobranchii . . . 397
      c. Teleostei . . . 397
III. Morphological Color Changes . . . 398
  A. The Effects of Backgrounds . . . 398
    1. White Backgrounds . . . 398
    2. Black Backgrounds . . . 399
  B. The Effects of Illumination . . . 399
  C. Relation to Physiological Color Changes . . . 400
IV. Significance . . . 401
  References . . . 401

## I. INTRODUCTION

The varied colors and patterns of fishes are mainly dependent upon multitudes of pigmented integumentary cells known as chromatophores. With some exceptions, these chromatophores are capable of changing their appearance, and thus the aspect of the fish as a whole is altered. Chromatophores are thus specialized effectors.

Certain chromatophores (iridophores) alter their color, but the activity of most is characterized by a shift in the position of the contained pigment, so that more or less is exposed to view. By these means changes in shade, hue, or even pattern are produced, and since the chromatophores respond rather promptly the effects are easily noticeable and often striking.

In addition to changes of this sort, the appearance may also be altered by other means. Either the number of chromatophores or the amount of pigment which they contain may increase or decrease. Such modifications of the basic pigmentation are slow, gradual, and inconspicuous, but the final result may be more marked than that achieved by pigmentary migrations.

Because of the differences in the time required, it is natural that the changes brought about by the characteristic responses of the chromatophores should have received more attention than those which arise from the quantitative changes in pigmentation. The distinction between these two types of color changes has long been recognized, however. In 1909 Sećerov proposed that the color changes which arise from the specific behavior of the chromatophores be termed physiological, and those which depend on alterations of the basic pigmentation be designated morphological.

It is convenient to discuss the two types separately and since a knowledge of physiological color changes contributes to the understanding of morphological changes they will be dealt with in that order.

Since citation of specific sources for all the statements made in this article would require a very extensive bibliography, references to individual contributions have been limited to a few papers published since 1943. The earlier publications cited are reviews of color changes in animals and these should be consulted for further details and for references. The reviews by Fuchs (1914) and Parker (1948) together provide a bibliography of about 1700 contributions published prior to 1943.

## II. PHYSIOLOGICAL COLOR CHANGES

### A. Chromatophoral Responses

### 1. Pigmentary Migration

*a. Nature.* The pigment within most chromatophores varies in its position. It may appear as a solid, rounded mass in the center of the cell, so that the processes are colorless and indistinguishable. At other times the pigment may be distributed throughout the cell, revealing its finest branches.

It was long supposed that these states resulted from the withdrawal or extension of the processes of the chromatophore, much as in the case of pseudopodia. This has been observed in undifferentiated chromatophores, but careful study of established chromatophores has shown that the movements of the pigment are generally independent of changes in the shape of the cell.

In accordance with this, the terminology applied to these activities has been revised. It was formerly customary to speak of the contraction and expansion of chromatophores, or of contracted and expanded chromatophores. It is preferable to speak of the aggregation (or concentration) of the pigment when it moves toward the center of the cell, and of the dispersion (or distribution) of the pigment when it moves outward. The final state is described as aggregated or dispersed as the case may be.

The movements of the pigment may be arrested at any intermediate stage and remain so for long periods. When it again shifts, it may move in either direction. Occasionally clusters of pigment granules may move with a certain degree of independence, and concentration and dispersion of pigment has been observed within a process detached by microdissection.

*b. Time required.* The time required for normal movements of pigment from one extreme to the other is highly variable. It may be measured in seconds, minutes, hours, or days. In *Crenilabrus* the shift occurs in a few seconds; in *Fundulus* 1 to 2 minutes are required; in *Ameiurus* from 1 to 3.5 hours are necessary; and in *Anguilla* it has been reported that 20 days are needed. Chromatophores in isolated tissues often respond to treatment with hormones or drugs more promptly than they do while still controlled by the animal. The time needed for the controlling mechanism to act may thus conceal the rapidity with which the effector can actually respond. In the case of melanophores the time required for concentration of pigment is generally longer than that for dispersion, but the reverse is true in *Lebistes*. For erythrophores, dispersion is usually the slower process.

*c. Pulsation.* By immersing isolated scales from various teleosts in suitable salt solutions a cyclic activity of chromatophores can often be produced. This is termed pulsation. The time required for the pigment to complete its cycle of dispersion and concentration is about 1 minute. This affords a convenient opportunity to study the details of pigmentary migration and throws light on other aspects of chromatophoral behavior.

*d. Rest and activity.* The relation of pigmentary movements to states of rest and activity have been variously interpreted. The earlier investigators, influenced by the fact that nervous stimulation caused concentration of melanophore pigment, regarded this as the active state. Subsequently, the opposite view found a few supporters, but both of these ideas were largely replaced by the hypothesis that chromatophores in which the pigment is intermediate in position (stellate) are in a passive state, while those in which the pigment is either dispersed or aggregated are active.

Rather than attempt to equate rest and activity with accomplished states, some workers have preferred to regard the pigmentary movements themselves as the important element. According to their view, a chromatophore is to be regarded as active if the pigment is shifting its position; if it is quiescent, the chromatophore may be considered at rest. The actual position of the pigment is immaterial.

*c. Fatigue.* Some efforts have been made to elicit fatigue in the responses of chromatophores. Since they may remain indefinitely at any stage of concentration or dispersion, the problem was approached by subjecting fishes (*Fundulus*) to alternating stimuli occurring at intervals adjusted to produce repeated concentration and dispersion of pigment. After nearly 1500 successive changes over a period of 48 hours no evidence of exhaustion appeared. The shifts were carried to completion, and the time required was even slightly less than before the trial was begun.

There are numerous reports of instances in which the rapidity of pigmentary responses has increased with repetition. These have been interpreted as facilitation and as learning, but there is some evidence for the belief that such a result generally occurs after periods of pigmentary immobility and represents a gradual return to the usual rate of migration.

## 2. PHYSICAL COLOR CHANGES

In addition to chromatophores in which movements of the pigment content occur, there are others which produce astonishing effects by entirely different means. They are known as iridophores, or iridocytes, because of their iridescent appearance, and may be found in a variety of teleosts. They contain several elongated crystals, presumably of guanine, which reflect light of varying wavelengths. They are commonly associated with other chromatophores to form compound chromatophores, but the components of such a structure retain their individual characteristics.

In *Fundulus* the light reflected from the crystals of different iridophores may be of various colors, but a single iridophore may produce each of these colors in turn. When changes occur the colors appear in spectral sequence, passing from bluish green through yellow and orange to red within 5 seconds. During recovery, which requires from 40 seconds to two minutes, the same colors appear but in reverse order. Following this there is a period of from 15 to 20 minutes during which the iridophore fails to respond.

In *Holocentrus* the iridophores contain two or three needle-like crystals which are generally purplish red. When an isolated scale is transferred from sea water to certain salt solutions, the iridophores become deep sky blue, next pale blue, then pale yellow, and finally colorless. If the scale is returned to sea water, the purple color of the iridophores is restored after the colors mentioned have appeared in reverse order. These alternating changes may be repeated for several hours. During the changes in color the crystals do not change their position, even by rotation, and no other significant events have been observed. It has been suggested that minute changes in the thickness of the laminae of the crystals are responsible for alterations in the interference patterns which are presumably responsible for the colors displayed.

## B. Modes of Control

The methods by which chromatophoral responses are controlled and coordinated are in general use in the body. Early investigators were able to demonstrate the effectiveness of the nervous system in some animals, though in others the chromatophores showed a puzzling independence. Following the discovery of hormonal communication this mechanism was soon shown to provide an explanation for many of these exceptions. Finally, the demonstration of chemical mediation of the nervous impulse provided an additional means of interpretation for situations which other concepts failed to illuminate. In fact, investigations of chromatophoral activities have contributed much to our knowledge of the actions of these various agents and their integration into harmonious systems.

If one accepts the idea that chemicals discharged by the terminations of axons supplying the chromatophores are responsible for their activation, one distinction between neural and hormonal control disappears. Two other considerations nevertheless make it desirable to differentiate between these methods. One is that responses involving efferent nerve fibers generally appear more promptly than those produced by hormones. The other is that some animals depend largely or entirely on one method, some rely on the other, and still others utilize both. Examples of each will be discussed after the agents themselves have been examined.

### 1. HORMONAL CONTROL

*a. Pituitary hormones.* The pituitary gland enjoys the same preeminence in the hormonal control of color changes that it does in its relations to other endocrine glands. If it is removed (hypophysectomy) the pigment within the melanophores usually becomes aggregated and a persistent pallor is produced. This can be temporarily overcome by the injection of pituitary extracts which bring about dispersion, but normal color changes are abolished. In some animals, however, the effects of hypophysectomy or of injections of pituitary extracts are negligible. There are also cases in which removal of part of the pituitary produces darkening, and injections of extracts causes aggregation of melanophores.

These different effects have caused considerable perplexity and dispute, but it now seems probable that there are two explanations of the discrepancies. One is that melanophores of some fishes respond differently or not at all; the other is that more than one hormone is involved.

The most important pituitary hormone in most fishes, as far as color changes are concerned, is intermedin. This hormone has in fact been termed the chromatophorotropic principle of the gland. It is present in animals which are insensitive to its effects and even in many which lack responsive chromatophores. Its characteristic effect is the dispersion of melanophore pigment and a consequent darkening of the animal. Its absence results in aggregation of pigment and pallor. Intermedin has no evident effect on *Fundulus* melanophores, however, unless they have been denervated. In *Pseudopleuronectes* neither innervated nor denervated melanophores respond.

Another pituitary hormone has proved so elusive that it has usually been designated as W-substance. For a time it was hardly more than a hypothetical antagonist of the B-substance, intermedin, and therefore a cause of melanophore aggregation and pallor, yet a number of investigators who worked on *Fundulus* between 1918 and 1933 reported that pituitary extracts, among them an anterior lobe extract ("Antuitrin"), caused aggregation of melanophores. During this same period similar observations were reported for *Phoxinus*, *Gobius*, and *Pleuronectes*.

A few years later it was demonstrated that the removal of the anterior lobe of the pituitary from various sharks and rays was followed by persistent dispersion, but attempts to obtain further evidence were not particularly successful.

More recently, however, it has been reported (Healey, 1948) that in *Phoxinus*, extracts of pituitaries from trout, *Ameiurus*, and *Phoxinus* itself cause melanophore aggregation, both in normal fishes and those in which the spinal cord has been severed. In *Phoxinus* the hormone responsible for this aggregation appears to originate in the anterior lobe. It is interesting that extracts of *Ameiurus* pituitaries disperse *Ameiurus* melanophores but aggregate *Phoxinus* melanophores, while extracts of *Phoxinus* pituitaries which aggregate *Phoxinus* melanophores produce dispersion in frog melanophores.

There is also evidence for the presence of intermedin in *Phoxinus*, because the removal of a small part of the pars intermedia interferes with dispersion. The melanophores are capable of reacting to intermedin, for injection of "Bayer-Hypophysin" and extracts of frog pituitaries cause some dispersion in spinal minnows.

The situation is still further complicated by a recent report (Enami, 1955) that the pituitary and the hypothalamus of the oriental catfish, *Parasilurus asotus*, contain a melanophore-aggregating hormone. Since hypophysectomy does not significantly reduce the potency of hypothalamic extracts, while lesions of the median eminence and section of the pituitary stalk reduce the amount of the hormone in the pituitary itself, it is suggested that the hormone is produced in the hypothalamus and transported to the pituitary for storage. (The pituitary also produces intermedin.)

It can only be concluded that the identity of the "W-substance" is still a problem for further research.

*b. Adrenaline.* This hormone, the first to be identified with chromatophoral responses, exerts a powerful aggregating effect on melanophores and therefore causes pallor. It excels intermedin both in potency and in the universality of its effectiveness, though the melanophores of *Parasilurus* are reported to be insensitive to it. In spite of these characteristics, there is little reason to believe that blood-borne adrenaline is an important factor in ordinary chromatophoral activation.

There is one response in which it may nevertheless play a part. Many fishes when alarmed or frightened show a rapid and extensive aggregation of melanophores resulting in what is commonly termed "excitement pallor." In *Ameiurus* and *Pseudopleuronectes* this may be seen in denervated areas; consequently a hormone appears to be involved though its source remains uncertain.

*c. Other hormones.* In addition to the hormones already mentioned, several others are known to have limited effects on chromatophores. Some fishes display color changes during the reproductive period. These may be induced out of season by the injection of appropriate gonadal hormones and may often be eliminated by castration.

Thyroxine sometimes produces aggregation in melanophores, as well as definite modification of the pigmentation.

These hormones play little or no part in ordinary chromatophoral activity, but instead are directed toward the production of morphological color changes.

### 2. Neural Control

In many fishes, but not in all, it is a simple matter to cut the nerves leading to a particular region and thus to produce an area in which the responses of the chromatophores are greatly affected. In light fishes, a dark band or area is produced as a result of the dispersion of pigment in the melanophores. This persists for some time but gradually fades. Since this method was first used numerous variations and refinements have been devised and it has proved extremely useful. It is also possible to demonstrate by histological methods that the chromatophores of some fishes are abundantly supplied with nerve endings.

The earlier investigators naturally applied the conventional concepts of neural physiology to the interpretation of their observations. As information accumulated, however, numerous phenomena which could not be explained by the older theories were discovered. Accordingly, when the humoral theory of nervous activation was proposed experiments were soon devised to show whether or not it might be applied to chromatophoral activities. The neurohumoral theory, which was largely developed by Parker and is best summarized in his publications, resulted from such studies. It did not meet with complete approval, but it provides an explanation for many puzzling situations and is a well-recognized doctrine of chromatophoral physiology.

*a. The neurohumoral theory.* This theory, briefly stated, holds that the terminations of the neurons which supply the chromatophores produce chemical substances termed neurohumors which activate the chromatophores. It is also maintained that there are two kinds of nerve fibers with antagonistic effects. One type of fiber produces a neurohumor which causes pigment dispersion and the other a neurohumor causing aggregation.

The neurohumors appear to be the ones which are known to be involved in the operation of the autonomic nervous system. One of these is acetylcholine. Since this is rapidly inactivated by cholinesterase, it is necessary to block the action of this enzyme before consistent results can be obtained. When this is done it has been found that acetylcholine causes dispersion in melanophores, though in *Parasilurus* it is said to produce aggregation. It is present in the darkened skin of some fishes in concentrations within the limits known to be effective when injected. It has not been demonstrated that it is absent when the skin is blanched, nor has it been proven that it is produced by nerve fibers, but the evidence available is suggestive.

The other neurohumor is a substance similar to, or identical with, adrenaline. It produces aggregation in melanophores. Some investigators have identified this neurohumor with sympathin, which Cannon regarded as the product of the sympathetic nerve endings. The presence of adrenaline has been demonstrated in the skin of pale catfishes, but it has not been proven that it was derived from aggregating nerve fibers

A third neurohumor has been extracted from the skin and fins of *Mustelus* when the animal is extremely pale. It is not adrenaline, though it has the same effect on melanophores. It was termed selachine by Parker, who performed the series of experiments in which it was obtained.

*b. Nature of chromatophoral nerve fibers.* Inasmuch as some of these neurohumors are substances associated with the autonomic nervous system, it might be supposed that the nerve fibers supplying the chromatophores belong to this system. In this connection it is interesting that various drugs which affect the autonomic system, or produce results which correspond to those arising from autonomic stimulation generally have chromatophoral effects.

Nevertheless, recent studies of the autonomic system suggest that the distinctions between sympathetic and parasympathetic fibers, and possibly those between autonomic and somatic efferent fibers, are much less real than they were previously believed to be. Until more complete information concerning the autonomic nervous system of fishes is available the nature of the fibers supplying the chromatophores must be regarded as uncertain.

### 3. RECEPTORS

With the exception of direct chromatophoral responses elicited by stimulation of the chromatophore itself, color changes are generally produced by the action of the nervous and endocrine agents just enumerated following the stimulation of appropriate receptors. The receptors which are most intimately concerned are those which are sensitive to light. In order of importance they are the eyes, certain portions of the central nervous system, and the skin.

*a. The eyes.* The importance of the eyes is easily demonstrated by the effects of blinding, which naturally abolishes the responses ordinarily induced by changes in the shade, color, or pattern of the surroundings. Generally a blinded fish becomes dark and remains so while it is illuminated regardless of changes in background (e.g. *Ameiurus*). *Rhombus* (= *Scophthalmus*) and certain other flatfishes are said to become intermediate or variable in shade though other flatfish genera (*Rhomboidichthys, Lophopsetta*) are reported to become dark. A number of fishes respond to changes in illumination after blinding and these are discussed below.

The special importance of the eyes lies in their ability to form an image of the environment on the retina. An impression of the color, shade, and pattern of the background is thus produced in the central nervous system. In addition, the ratio of the illumination reflected from the background to that coming directly from the source may be evaluated. This ratio, which is termed the albedo, is of great importance. Indeed, it is generally believed that the dorsal and ventral halves of the retina are functionally specialized, and some histological differences have been found in *Fundulus*.

*b. Central nervous organs.* The fact that various blinded fishes continue to respond to changes in illumination indicates the presence of photic receptors other than the eyes. In some cases in which this has been demonstrated, including two species of *Gobius*, *Ctenolabrus*, *Lepadogaster*, and *Fundulus*, no effort was made to identify the organ or organs involved, but in other cases various parts of the central nervous system have been tested.

In *Lampetra planeri* illumination of the head after removal of the eyes is effective in producing swimming or burrowing movements. These also persist after removal of the pineal complex, and it is accordingly supposed that other tissues of the brain are sensitive to light. The spinal cord has also been shown to be affected if the pigmented coverings are partially removed. *Lampetra* does not respond to backgrounds, but it does display a cyclic change, becoming dark during the day and pale at night. If the pineal complex is removed these rhythmic changes are disturbed in adults and abolished in the ammocoete larvae. They are also abolished in adults if both eyes and pineal complex are removed.

In *Phoxinus* responses to illumination occur after removal of both the pineal body and the eyes, and the sensitivity of other diencephalic structures, possibly the ependymal lining, has been postulated to account for this.

In *Ameiurus* removal of the pineal body also fails to abolish the pigmentary responses of blinded fishes, but in this case the search for a suitable receptor led to the skin, which will be considered next.

*c. The skin.* The skin has often been suspected of responding to illumination by direct or reflex activation of the chromatophores. A choice between these possibilities is difficult to establish, but in *Ameiurus* there is evidence to show that a reflex is involved. In this fish the melanophores of blinded individuals become aggregated when the fish is placed in darkness, but if they are in a denervated area the melanophores fail to respond.

In general it may be concluded that responses to backgrounds depend on visual stimulation, but responses to illumination may be initiated by the skin, the pineal complex, and possibly by other nervous structures not yet identified.

## 4. Comparative Account of Chromatophoral Control

*a. Cyclostomata.* The few studies of the responses of these fishes indicate that they are entirely independent of efferent nerve fibers.

Neither stimulation nor cutting of nerves produces any effect. In *Lampetra* it appears that the simple responses which occur are produced by varying the concentration of a single hormone, intermedin, which produces dispersion of melanophores. The activity of the pituitary is apparently controlled by the pineal complex and the eyes.

The responses observed in *Myxine glutinosa* seem also to be determined by a single hormone, though it is reported that a process other than pigmentary migration occurs.

*Petromyzon marinus* is said to exhibit very striking color changes which would seem to demand a more complicated means of control, but details are not available.

*b. Elasmobranchii.* In this group responses are also simple and appear to be largely regulated by intermedin, but it will be recalled that there is evidence for the presence of an aggregating hormone in the anterior lobe of the pituitary of a number of members of this group.

Some studies of *Mustelus canis* and *Squalus acanthias* have indicated the presence of nerve fibers which cause aggregation in melanophores. These have been suggested as the source of the neurohumor selachine.

*c. Teleostei.* The larger variety of chromatophores and the more complicated responses exhibited by many bony fishes is associated with the development of more varied mechanisms of control, and with the increased importance of the nervous system.

*Fundulus* has a particularly simple type. The essential agents appear to be opposing sets of nerve fibers which secrete neurohumors. Intermedin is effective only on denervated melanophores, and hypophysectomized individuals show normal responses. The aggregating effects of pituitary extracts observed in the early days still remain unexplained.

In *Anguilla* intermedin is of great importance, and an aggregating hormone from the pars tuberalis of the pituitary has been described. There are reported to be antagonistic sets of nerve fibers present, but they are of slight significance.

The color changes of *Ameiurus* depend on the same agents as in *Anguilla*, except that there is no evidence for an aggregating hormone. Nerve fibers play a greater role.

The responses of flatfishes (*Paralichthys*, *Pleuronectes* and *Pseudopleuronectes*) are particularly interesting because of the local responses which some of them exhibit. In addition to typical background responses, light or dark spots may appear and disappear. Excitement results not only in pallor in dark individuals, but also in darkening in pale ones; the spots may become more or less conspicuous.

The rapidity and specificity of the responses indicate the importance of nervous control, and cutting of nervous pathways produces definite effects. Nevertheless, excitement will cause pallor in dark denervated areas though not as promptly as in the rest of the body. This effect can be simulated by injections of adrenalin, and it seems necessary to assume that some such aggregating hormone is responsible.

The injection of intermedin is entirely without result, whether the nervous system is intact or not, but there is some evidence that points to the possibility that some dispersing hormone is present.

## III. MORPHOLOGICAL COLOR CHANGES

During the development of the characteristic color pattern of the adult, a fish may undergo a series of modifications of its basic pigmentation. These may accordingly be regarded as morphological color changes. Alterations of this type are largely the expression of the operation of genetic factors, and this aspect of the subject will not be discussed here. The so-called nuptial changes associated with reproductive activity, and also dependent on intrinsic factors, must also be passed over.

In addition to these changes, however, the pigmentation of both young and adult fishes may be altered by the action of stimuli from the environment, and this type of morphological color change is briefly described.

### A. The Effects of Backgrounds

#### 1. White Backgrounds

If fishes which respond to a white background by concentration of pigment in their melanophores are exposed to its influence for protracted periods they eventually become even paler than they were when the original physiological response was completed. If the melanophores are counted from time to time a decrease in number is soon apparent. The extent of this decrease varies in different species, and in different individuals of the same species. In an experiment with *Fundulus heteroclitus* one-third of the melanophores disappeared within 29 days. Seven individuals observed for 69 days lost 59% of the melanophores, and in one fish 89% disappeared. In short experiments at 21° C. the rate of decrease is about 1% per day.

The decrease in pigmentation may also be detected by extracting melanin from the bodies of fishes and comparing the extracts with others from suitable controls.

Decreases in the number of melanophores are apparently the result of their degeneration. Irregular masses of pigment appear in the locations which they previously occupied. Later this pigment shifts its position, probably because it is ingested by phagocytes, and much of it is lost at the surface of the body. The possibility that the melanophores may discharge their pigment and still persist unseen has, however, not been eliminated.

When young fishes are reared on white backgrounds the development of melanophores is greatly retarded, but degeneration is not a significant factor.

### 2. Black Backgrounds

Fishes kept on black backgrounds but otherwise under conditions like those in the experiments just mentioned show no such decreases. Instead the number of melanophores remains unchanged or increases. *Fundulus* shows little change unless the pigmentation has previously been reduced by a sojourn on a white background. In other fishes, such as *Lebistes,* substantial increases occur.

Increases in number presumably arise by the proliferation and differentiation of prospective pigment cells. Possibly mitotic division of the melanophores themselves may be of some importance.

Young fishes reared on black backgrounds become much darker than individuals in normal surroundings and the melanophores are correspondingly more numerous.

As the foregoing experiments suggest, visual stimuli are of paramount importance, as they are in physiological color changes. All available evidence supports the conclusion that the albedo is again the determining factor, and that illumination is effective only in conjunction with background stimuli or their equivalent.

## B. The Effects of Illumination

If two specimens of *Ameiurus*, one of which has been blinded, are kept for a long period in an illuminated white bowl, the normal fish becomes extremely pale but the blinded fish becomes sooty black. The difference must be attributed to the blinding. If such a blinded fish is kept in darkness, however, reductions in pigmentation occur which are due to lack of illumination. Reductions in pigmentation may be produced in *Fundulus* kept in darkness, even in normal fishes, but they are slight.

Furthermore, it has occasionally been reported that fishes exposed to light from below for long periods show increased pigmentation on the under side. The circumstances of the early experiments made it difficult to appraise their results, but more recent studies have confirmed the fact that illumination is effective under proper conditions. Thus if *Ameiurus* or the flounder *Paralichthys* is kept in an aquarium having black sides and top and an illuminated glass bottom abundant melanophores appear in areas normally unpigmented. This also happens if blinded fishes are illuminated from below, regardless of the surroundings. If the sides and top of the aquarium are white, normal fishes show no such change.

These results are explained by the observation that illumination produces increased pigmentation on the exposed surface only when all circumstances combine to produce dispersion of pigment in the melanophores. This finding provided additional support for the concept of the relation between morphological and physiological color changes set forth in the next section.

### C. Relation Between Morphological and Physiological Changes

Alterations in the basic pigmentation such as those just described have long been attributed to the influence of the temporary responses of the chromatophores themselves. A number of investigators during the past fifty years have noted that increases in pigmentation are preceded by a period in which the pigment in the chromatophores concerned is dispersed, while decreases are foreshadowed by aggregation. Accordingly, several variations of the hypothesis that the physiological color changes are the causes of the subsequent morphological changes have been proposed. The earlier expressions of this idea were often stated in terms of theories of rest and activity, of innervation, and of trophic influences which have come to be regarded as inadequate if not erroneous. The concept itself has persisted, however. In a more recent form, it may be expressed as follows; any conditions which bring about the concentration of pigment in the central part of the chromatophore will, if maintained, inhibit or retard their further development or even bring about the destruction of some of those already present; conversely, any conditions which lead to the dispersion of pigment throughout the chromatophore will, if maintained, promote the development of chromatophores or insure their continued existence. According to this view, morphological color changes are not regarded as dependent upon physiological color changes. Instead, they arise from the operation of the agents which produce those changes and accentuate their effects.

Thus either hormones or neurohumors could be responsible for morphological color changes. The significance of neurohumors has yet to be demonstrated but it has been shown that if *Ameiurus* is hypophysectomized as well as blinded, the increase in pigmentation caused by illumination in blinded fishes fails to occur, and melanophore degeneration sets in. The dispersing hormone is essential for the development and maintenance of melanophores.

It has also been demonstrated (Robertson, 1949) that administration of thyroid extracts to trout parr (*Salmo gairdnerii*) induces the appearance of smolt coloration through the destruction of melanophores and increased development of guanine.

## IV. SIGNIFICANCE

Color changes are only a means of adjusting the appearance of the fish to the environmental situation in which it finds itself. The significance of these changes is therefore only one aspect of the significance of animal coloration in general. It has been interpreted as the secondary result of excretory deposition and as a means of absorbing or excluding radiant energy. The best known and most discussed interpretation is the theory of concealing coloration, which maintains that the animal is rendered less conspicuous in its surroundings. The profound influence of the background speaks in favor of this view, and there is some experimental evidence to support it. If fishes adapted to different backgrounds are placed together in a tank where they are subjected to the predations of birds and other fishes, those which are best adapted to the color of the test tank are least likely to be captured.

The pigmentation of fishes probably has a variety of advantages which vary in importance in different species and even at different times in the life of a single individual. The changes which occur confer the benefits of adaptability and thus perfect the relation of the animal to its environment.

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CHAPTER IX

# WATER QUALITY REQUIREMENTS OF FISHES AND EFFECTS OF TOXIC SUBSTANCES

PETER DOUDOROFF

| | | Page |
|---|---|---|
| I. | Introductory Discussion | 403 |
| II. | Temperature | 406 |
| | A. Resistance and Acclimatization to Heat | 406 |
| | B. Resistance and Acclimatization to Cold | 408 |
| III. | Salinity and Osmotic Pressure | 409 |
| IV. | pH and Dissolved Carbon Dioxide | 411 |
| | A. Ranges of Tolerable pH | 411 |
| | B. Resistance to Carbon Dioxide | 411 |
| V. | Dissolved Oxygen and Nitrogen | 413 |
| | A. Dissolved Oxygen Deficiency | 413 |
| | B. Oxygen and Nitrogen Excess (Supersaturation) | 415 |
| VI. | Suspended Solids (Turbidity) | 416 |
| VII. | Miscellaneous Toxic Substances | 417 |
| | A. Variety, Sources, and Literature | 417 |
| | B. Mode of Action and Penetration of Toxicants | 418 |
| | C. Antagonism and Synergism | 420 |
| | D. Toxicity Bioassays | 420 |
| | E. Strong Alkalis and Acids | 421 |
| | F. Ammonia and Ammonium Compounds | 422 |
| | G. Weak Acids and Inorganic Sulfides | 422 |
| | H. Cyanides and Metal–Cyanide Complexes | 423 |
| | I. The Metals as Salts | 424 |
| | J. Other Toxic Water Pollutants | 426 |
| | References | 427 |

## I. INTRODUCTORY DISCUSSION

Natural waters have widely varying physical and chemical properties. The suitability of any water as a medium for fish depends on its temperature and the concentrations of dissolved atmospheric gases, salts, and other minerals. It depends also on the state of adaptation of the fish to these environmental conditions. The contamination of inland and coastal waters with various industrial and domestic wastes often has rendered these waters unfit for susceptible fish species. This chapter is an introduction to the physiological and toxicological principles of water quality appraisal relating to the requirements of fishes, and a guide to correct interpretation and application of basic data to be found in the voluminous pertinent literature. All of the essential information could not be included. Reference is made, however, to some useful literature reviews and compendia, as well as original sources of data.

Simple physical and chemical criteria of the suitability of waters for fish cannot be prescribed for general application, because the requirements of species vary greatly, and also because of the large number and the complex interaction of factors which together determine or can influence the quality of water. A dissolved oxygen concentration which is inadequate and fatal for some fish at ordinarily favorable temperatures may be tolerated by them indefinitely at extremely low temperatures. Concentrations of some toxic substances which are rapidly lethal in ordinary fresh waters may be harmless when these substances are added to unusually hard and alkaline waters. Thus, there is wide disagreement between the results of investigators who have evaluated the resistance of fish to the same harmful agents under different experimental conditions. Arbitrary water quality standards and lists of unqualified critical or maximum tolerable concentrations of water pollutants which can be harmful to fish have been published repeatedly. Useful though these may appear to be, they actually are apt to be misleading more often than helpful, for they are based on fragmentary information and have been prepared without proper regard to unrefuted contradictory evidence. An intelligent approach to water quality and pollution problems relating to the complicated requirements of aquatic life involves careful consideration of all available pertinent data, in the light of general principles discussed herein.

A distinction must be made between tolerable environmental conditions determined experimentally in the laboratory and those conditions under which fish can be expected to occur and thrive in nature. In order to maintain themselves in their natural habitats, fish must be more or less active, they must find and capture food (competing effectively with other species), resist currents, escape enemies, grow, and reproduce successfully. Their ability merely to survive under unnatural experimental conditions requiring no sustained activity obviously is not a reliable indication that the quality of the medium is satisfactory. For example, it is generally known that so-called warm-water fishes can live indefinitely at low temperatures to which they are normally exposed in winter, but do not thrive in waters which remain cold throughout the year. Because of depressed metabolic rate, they are not active and grow little or not at all at the low temperatures, and successful spawning and development may be impossible. On the other hand, cold-water fishes, such as trout, may be unable to compete successfully with warm-water species in environments in which summer temperatures are higher and dissolved oxygen concentrations relatively low, though not intolerable. Graham (1949) has presented evidence that brook trout, *Salvelinus fontinalis*, are unable to take up the oxygen required for maximum sustained activity, and their maximum steady cruising speed may be markedly reduced, when the dissolved oxygen falls to a level well above the lower limit of tolerance and not far below the air-saturation level. According to Fry and Hart (1948), at moderate temperatures the maximum rate of oxygen uptake of goldfish, which are warm-water fish, becomes dependent upon the oxygen tension only when the oxygen tensions are relatively low.

It is not known just how much sustained activity may be essential for the well-being of fishes in nature, and undoubtedly the requisite activity level varies greatly with the environment. A healthy fish population certainly can exist under conditions which are not conducive to maximum activity and rapid growth. Critical environmental conditions rarely persist in fish habitats for long periods of time, and it is not often possible to determine which factors are primarily responsible for the absence of certain fishes in unstable aquatic environments which are not obviously unsuitable. The inconclusiveness of field studies is well exemplified by their failure to provide a truly firm foundation for decisions concerning minimum dissolved oxygen concentrations necessary for the maintenance of normal, healthy, fish populations.

Ellis (1937), reporting results of very extensive field observations, stated that good, normally varied, warm-water fish faunas were not found in polluted waters containing less than 4 parts per million dissolved oxygen, and were found only rarely in waters with an oxygen content less than 5 parts per million (p.p.m.).[1] He concluded that 5 p.p.m. probably is the limiting or critical level. However, satisfactory evidence that dissolved oxygen concentrations much lower than those recorded did not occur at any time in the waters sampled was not presented. Furthermore, Ellis was unable to prove that reduction of dissolved oxygen was primarily responsible for the elimination of some desirable fish species from waters excessively contaminated and enriched with organic wastes and not any other associated factor, such as excessive multiplication of saprophytic and other pollutional organisms which may render an environment unfit for the normal clean-water fauna. Katz and Gaufin (1953) and others have reported the occurrence of varied warm-water fish populations in waters in which widely fluctuating dissolved oxygen concentrations sometimes fell well below 4 p.p.m. (e.g. to 1.6 p.p.m.) for short periods. Evidence was presented that the adverse influence of oxygen-depleting organic pollutants, such as domestic sewage, on the fish fauna of a stream can be most severe or extensive when temperatures are low and dissolved oxygen concentrations remain relatively high.

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[1] A *part per million* by weight (p.p.m.), the unit of concentration commonly used by sanitary water chemists and others in the United States, and used accordingly throughout this chapter, is practically equivalent to one *milligram per liter* of solution when the specific gravity of the solution (or water) is very near unity. Although it is not always immaterial, the difference between these two concentration units may be disregarded by those readers of this chapter for whom milligram per liter is a more familiar expression than p.p.m.

The observation of Ellis (1937) that various freshwater fishes may show respiratory compensations when the dissolved oxygen in the water is reduced only a little below 5 p.p.m. is not coupled with any evidence that such compensation is indicative of serious impairment of any vital function or activity. The avoidance by fishes of waters of altered quality in experimental gradients sometimes has been regarded as a reliable indication of adverse conditions. This view appears to be unfounded. Doudoroff (1938) found that greenfish, *Girella nigricans*, selected in a horizontal gradient high temperatures which are quite unusual in their native marine habitat. Acclimatization to different temperatures had some temporary influence on the reactions, but normal habitat temperatures were definitely avoided by greenfish acclimatized thereto. Avoidance reactions of fishes in natural gradients may limit dispersal, but their close correspondence with reactions to much steeper artificial gradients obviously may not be assumed. Fish in experimental gradients have been unable to avoid rapidly fatal concentrations of some toxic substances, such as ammonia and certain heavy metals.

It must be concluded that the present state of our knowledge and understanding of the water quality requirements of fishes is such that the suitability of any untried water must be judged chiefly with reference to limits or ranges of tolerance determined experimentally. Even these cannot be precisely defined without much qualification. One can only surmise what alterations of water quality will have no detrimental effects on populations of fishes in their natural environments. The lethality data presented in connection with the following discussion of the resistance of fish to various harmful agents should be viewed in the light of the foregoing considerations, and applied accordingly.

## II. TEMPERATURE

### A. Resistance and Acclimatization to Heat

The resistance of fishes to high temperatures varies markedly with their thermal history. The highest temperatures to which different species can be gradually acclimatized (median values) have been found to be about 41° C. for the goldfish *Carassius auratus* (Fry, Brett, and Clawson, 1942); 37.5° C. for the bullhead *Ameiurus nebulosus* (Brett, 1944); 33.2 to 33.3° C. for two cyprinids (*Pimephales, Hyborhynchus*), and 29.3 to 31° C. for four other North American cyprinids (*Rhinichthys, Semotilus, Notropis* spp.), the sucker *Catostomus commersonnii*, and the perch *Perca flavescens* (Hart, 1947); 31.4° C. for the marine greenfish *Girella nigricans* (Doudoroff, 1942); 25.3° C. for the brook trout *Salvelinus fontinalis* (Fry, Hart, and Walker, 1946); and 23.8 to 25.1° C. for five species of Pacific salmon of the genus *Oncorhynchus* (Brett, 1952).

These are "ultimate upper incipient lethal temperatures" (Fry *et al.*, 1946; Fry, 1947), the highest temperatures that apparently could be tolerated indefinitely by about 50 per cent of the experimental animals after maximum acclimatization to heat. The heat-acclimated fish withstood brief exposures to considerably higher temperatures, sometimes without showing any distress. On the other hand, much less extreme high temperatures proved rapidly fatal after acclimatization to low temperatures. The limits of tolerance for the hardy goldfish and bullheads acclimatized to 20° C. were 34.8° C. and 33.4° C., respectively. The maximum temperatures tolerated indefinitely by some other fishes acclimatized to 20° C. did not prove lower, however, than the maximum temperatures tolerated after acclimatization to relatively high temperatures. In many cases only fish acclimatized to temperatures more than 10 or 15° C. below these ultimate tolerance limits had decidedly lower limits of heat tolerance. The limits reported for *Gambusia affinis* acclimatized to 35° C., 20° C., and 15° C. are 37.3, 37.3 and 35.4° C., respectively (Hart, 1952).

In some freshwater species, Hart (1952) observed considerable geographic, seasonal, and other variations of the resistance to heat (also to cold) of fish acclimatized to the same temperatures. Other species did not show corresponding variations. Differences of results obtained in summer and in winter may have been due to incomplete acclimatization. Some of the ultimate upper incipient lethal temperatures reported above evidently are considerably lower than the true maximum values, having been determined by Hart (1947) in winter.

Acclimatization to heat is not a very slow process. Studies of the rate of acclimatization of fish which had been transferred from moderately low temperatures to higher temperatures ranging from 26 to 30° C. (Loeb and Wasteneys, 1912; Sumner and Doudoroff, 1938; Doudoroff, 1942; Brett, 1944, 1946) have shown that increased heat resistance usually is acquired fairly rapidly at these high temperatures, although there may be a latent period of one day or longer during which virtually no change occurs. Most of the resulting increase of resistance was achieved within one to three days. When fish which had been acclimatized to the high temperatures are transferred to lower temperatures, little or no loss of resistance may be detectable after the first three days. Thus, a relatively brief exposure to nonlethal high temperatures, or intermittent exposure, can result in markedly increased resistance to heat, which is not lost readily on subsequent exposure to low temperatures. The prevalent notion that large and sudden temperature changes are often fatal to fish because of the initial shock involved is not supported by these experimental findings. Brett (1941) found that "tempering" of yearling brook trout by gradually raising the temperature of their medium over a period of only 15 minutes, in order to reduce the initial shock, did not produce any appreciable increase of their resistance to heat.

The speed of acclimatization to heat has been found to vary with temperature. Brett (1946) reported that the rate of gain of heat resistance of goldfish which had been acclimatized to different temperatures and then transferred to temperatures 8° C. higher increased markedly with increasing acclimatization temperature. Brett (1944) noted also that the normally rapid acclimatization of bullheads to high temperatures seemed to be interfered with by deficiency of dissolved oxygen in the water in which they were held during the acclimatization period.

### B. Resistance and Acclimatization to Cold

Most of the fishes commonly used as experimental material can withstand indefinitely temperatures near the freezing point of their medium, temperatures to which they are normally exposed in winter. Therefore, the susceptibility of fishes to chilling has not often been investigated intensively. However, Doudoroff (1942, 1945) has presented evidence that lethal cold can be more important than lethal heat as a factor limiting the distribution of marine fishes and as a hazard to some of these fish in their native habitats. Thermal history was shown to have a pronounced influence on resistance to chilling. The lowest temperatures to which the fish can be gradually acclimatized were not determined, but the experimental data reported indicate that some of the more susceptible marine species of the temperate zones cannot withstand 0° C. even after thorough acclimatization to very low (above zero) temperatures. Temperatures as high as 10 to 5° C. proved fatal to the species studied after acclimatization to 20° C.

Experiments on the rate of acclimatization of the greenfish *Girella nigricans* (Doudoroff, 1942) have shown that resistance to chilling is lost slowly on warming and is acquired no more rapidly on cooling. It was concluded that after any rise or fall of temperature, large or small, within the range of temperatures ordinarily encountered by the species in its natural environment, about one-half of the resulting change of resistance to cold occurs in the first 2 days, and nearly complete acclimatization is achieved only after about 20 days. Inasmuch as the gain of resistance to heat was much more rapid on warming, and its loss on cooling was very slow, the changes of resistance to heat and to cold with acclimatization evidently are more or less independent and distinct phenomena. In this connection, it is noteworthy also that the limits of cold tolerance of 20°-conditioned and 28°-conditioned *Girella*, based on 72-hour tests, differed by 5° C. (being 8 and 13° C., respectively), whereas the corresponding limits of heat-tolerance did not differ at all.

Some tropical freshwater fishes, as well as marine forms, are well known to be very sensitive to chilling. After acclimatization to high temperatures, temperatures well above 0° C. are fatal even to such cold-tolerant freshwater species as the goldfish (Fry *et al.*, 1942), the bullhead *Ameiurus nebulosus* (Brett, 1944), and the brook trout (Fry *et al.*, 1946). Young Pacific salmon of several species of the genus *Oncorhynchus* were unable to withstand 0° C. even after acclimatization to temperatures as low as 5 to 10° C. (Brett, 1952).

Death by chilling can be rapid at extreme low temperatures. At less extreme temperatures, death may be delayed for several days, the fish not being visibly affected during much of this time after they recover from initial shock effects, which seem to have no close relation to the final lethal action of the low temperatures. Doudoroff (1945) noted that the death of *Fundulus parvipinnis* at slowly lethal low temperatures is preceded by dehydration of tissues when the fish are in sea water, which is their normal medium, and can be delayed by dilution of the sea water with distilled water. Correspondingly, Brett (1952) found that young sockeye salmon were more resistant to slowly lethal low temperatures in dilute sea water than in fresh water. Osmoregulatory failure at these low temperatures is indicated.

Brief superficial freezing and supercooling (without freezing) to temperatures below the freezing point of body fluids have been tolerated by resistant species, but there seems to be no reliable confirmation of early reports of the survival of fish that had been frozen for a long time in ice, or frozen solid throughout in air (Borodin, 1934).

## III. SALINITY AND OSMOTIC PRESSURE

Inasmuch as osmoregulation is considered in Chap. IV, Vol. I, the adaptation of fishes to waters of different salinity is not discussed in detail here. It is well known that some so-called euryhaline fishes can live both in sea water, with a salt content near 3.5%, and in fresh water with very low dissolved mineral content, at least after gradual acclimatization to these extreme conditions; while other species are confined to a relatively narrow salinity range. The resistance of the latter species to variations of water salinity is not uniform. Such generalizations as the often repeated assertion of Ellis (1937) that "the limit of osmotic pressure tolerated in the external medium by fresh-water fishes" is "near 6 atmospheres, or the approximate equivalent of 7,000 p.p.m. of sodium chloride" apparently are without firm foundation. Ellis' conclusion is based largely on the findings of Garrey (1916), who noted correspondence between the freezing point depression of the bloods of freshwater fishes and that of the most saline medium (diluted sea water) tolerated by the minnow *Notropis blennius*. Doudoroff and Katz (1953) have pointed out, however, that limits of long-term salinity tolerance of different freshwater fishes below 4000 p.p.m. and above 14,000 p.p.m. of sea salts have been reported by other investigators. Corresponding osmotic pressures range from less than half to nearly twice the probable average osmotic pressure of the blood of freshwater fishes, which is equivalent to that of about 8000–9000 p.p.m. NaCl solutions. The very low salinities tolerated by some marine fishes which cannot live in fresh water indicate that the limits of salinity tolerance of marine fishes also are not strictly dependent on blood osmotic pressures.

The lethal action of some highly saline waters on freshwater fishes may be predominantly osmotic, and not referable to any one of several component salts or ions. The individual or specific toxicity of the different salts and ions, which is to be considered more fully later (p. 425), should not be overlooked, however. That of all the chlorides and all or nearly all the sulfates of the metals is referable chiefly to metallic cations, and not to the relatively harmless anions. Because of cation antagonism, the pronounced toxicity of simple solutions of single metal chlorides (including sodium chloride) consequently can be often greatly reduced by the addition of other metal chlorides (Garrey, 1916; Doudoroff and Katz, 1953). Sea water is a physiologically balanced solution of mixed salts, the individual toxicity of which is thus counteracted. Some highly saline natural waters differing markedly in their composition from diluted sea water with corresponding total salt content, such as those of the Quill Lakes of Saskatchewan described by Huntsman (1922), also support fish populations (freshwater forms). The chlorinity, the total salinity, and the osmotic strength of different natural brines and waters polluted with various saline industrial wastes certainly are not always adequate indices, however, of their relative suitability as media for fish.

The prevalent notion that distilled waters are soon fatal for freshwater fishes because of deficiency of dissolved minerals apparently is not well founded. Fish are extremely sensitive to some toxic contaminants, such as minute amounts of copper derived from stills, in waters with very low mineral content. Fishes have been held successfully for long periods, however, in pure distilled waters.

## IV. pH AND DISSOLVED CARBON DIOXIDE

### A. Ranges of Tolerable pH

A recent critical review of voluminous pertinent literature (Doudoroff and Katz, 1950) has led to the conclusion that most, if not all, fully developed freshwater fishes can live indefinitely in waters with pH above 5.0 and up to 9.0 at least. Much more extreme pH values, perhaps even below 4.0 and well above 10.0, also can be tolerated for long periods by the most resistant species, and for short periods by more sensitive forms. The pH of unpolluted fresh surface waters varies widely, depending chiefly on the concentrations of dissolved carbonates, bicarbonates, and free carbon dioxide, which are the principal buffer substances in natural waters. However, pH values outside the tolerable range and persisting long enough to be fatal to fish are very unusual. Acid and alkaline pollutants can have varying effects on fish, which may or may not be ascribable simply to the altered pH of the external medium. They will be considered mostly under the heading of toxic substances. Very young fish may be somewhat more sensitive to extremes of pH than adults. It has been reported that some freshwater fish embryos and larvae cannot live in waters which are only slightly alkaline, but the published evidence is inconclusive.

General conclusions relative to the pH requirements of marine fishes cannot be drawn from the very limited available data. The pH of sea water varies little, being usually between 7.5 and 8.5

### B. Resistance to Carbon Dioxide

Mortality of freshwater fishes in the absence of demonstrably toxic concentrations of dissolved (free) carbon dioxide has been ascribed repeatedly to variations of carbon dioxide tension or partial pressure. Powers (1938) claimed that "fish soon become deranged through compensating at short intervals for different $CO_2$ tensions" by readjustment of the alkali reserve of their blood. Fish mortality resulting from this "derangement" was said to occur, for example, when fish begin to feed at daybreak, coming to the surface repeatedly from water with a relatively high $CO_2$ tension. Powers (1937) also ascribed fish mortality in a slightly alkaline, soft water to abnormally low $CO_2$ tension. These conclusions are based on devious reasoning and questionable evidence, and they cannot be accepted without satisfactory experimental demonstration of their validity.

It has been established only that persistent high concentrations of free carbon dioxide can be decidedly injurious to fish because of its toxicity, or interference with respiration (Doudoroff and Katz, 1950; and Chap. I, Vol. I). Concentrations between 100 and 200 p.p.m. can be rapidly fatal to moderately susceptible freshwater fishes in well-oxygenated waters. Concentrations between 50 and 100 p.p.m. can cause immediate distress and may be lethal after more or less prolonged exposure, and they can prevent normal development. The ability of the fish to take up oxygen and to survive at low dissolved oxygen concentrations may be seriously impaired also. Free carbon dioxide concentrations above 20 p.p.m. occur rarely even in polluted waters. Fatal concentrations can result from the addition of acids stronger than carbonic acid to those natural waters that are very well buffered with bicarbonates (which seem to have no important direct influence on fish), but they do not persist long in waters exposed to the atmosphere. Much $CO_2$ can be liberated in some waters, or can be added thereto, without rendering the waters very acid. It can be fatal to fish in buffered solutions with entirely tolerable pH. Marked impairment of the ability of fish to utilize dissolved oxygen in some moderately acidified fresh waters (e.g. at pH near and above 6.0) doubtless has been ascribed erroneously to the influence of hydrogen ions (Wiebe *et al.*, 1934; Townsend and Cheyne, 1944). It is probably referable chiefly to the influence of free $CO_2$, the moderate reduction of the pH value of water through reduction of the total alkalinity without increase of the $CO_2$ tension having no such pronounced effect.

It has been reported that free $CO_2$ concentrations near 40 p.p.m. are rapidly fatal to young Pacific herring, *Clupea pallasii* (Shelford and Powers, 1915). Accordingly, these fish soon succumbed in sea water which had been rendered only slightly acid with an equivalent amount of sulfuric acid, liberating $CO_2$. However, both marine and freshwater fishes vary greatly in their resistance to $CO_2$. Doudoroff and Katz (1950) cite many pertinent references.

Fry, Black, and Black (1947) reported that the resistance of goldfish to carbon dioxide increased greatly with increasing temperature to which the fish had been thoroughly acclimatized. However, these authors determined and compared $CO_2$ tensions only, and not free $CO_2$ concentrations. Apparently there was little difference between maximum free $CO_2$ *concentrations* having no measurable influence on the ability of goldfish to reduce the oxygen content of water in sealed bottles at the different experimental temperatures. The data suggest, therefore, that free $CO_2$ concentration may be a more satisfactory measure of pollution of the external medium than is the $CO_2$ tension.

## V. DISSOLVED OXYGEN AND NITROGEN

### A. Dissolved Oxygen Deficiency

Depletion of dissolved oxygen in water, due to bacterial decomposition of putrescible organic matter and the respiration of various aquatic organisms, is often the cause of sudden mortality of fishes. Nevertheless, the dissolved oxygen concentration requirements of fishes (especially of marine fishes) have not yet been adequately investigated. Only a brief discussion of tolerance limits is presented here, inasmuch as the respiration of fishes in water is considered in Chap. I, Part 1, Vol. I.

It is now generally known that high temperatures, and also extremes of pH, high concentrations of free $CO_2$, and certain other toxic substances, can markedly increase the susceptibility of fish to deficiency of dissolved oxygen. It is apparent, however, that there are wide discrepancies between the tolerance limits reported in the literature and these must be ascribed to other factors, including differences of experimental conditions and techniques the probable import of which has been often entirely overlooked.

A number of determinations have been made of levels to which fish can reduce the dissolved oxygen content of water in small sealed containers before death intervenes, or up to the time when they lose equilibrium. Burdick *et al.* (1954) recently reported such experiments with several species of trout and with smallmouth bass at different temperatures, citing other pertinent references. Anoxial levels so determined obviously may not correspond to maximum dissolved oxygen concentrations that would be fatal to the experimental animals after indefinitely prolonged exposure, for the animals are exposed to these levels for a very short time only before they succumb.

Seeking to overcome this objection, Moore (1942) held various warm-water fishes for 24 hours at different levels within the thermocline of a thermally stratified lake with a vertical oxygen gradient. The numbers of fish surviving at the various dissolved oxygen concentrations and temperatures were recorded. Moore concluded that dissolved oxygen concentrations of at least 3.5 to 5.0 p.p.m. are essential to the survival of most warm-water fish for 1 day at summer temperatures (15 to 26° C.). He cited results of earlier work similar to his own to support this surprising conclusion, which is not in accord with many laboratory and field observations. His fish probably had been subjected suddenly to oxygen concentrations far below those to which they were accustomed, so that there was no opportunity for gradual adaptation. Other sources of error also may be involved.

Shepard (1955) presented evidence that the resistance of brook trout can be increased markedly by acclimatization, reducing the lethal level to little more than one-half the corresponding value for trout accustomed to air-saturated water. There is other evidence of such adaptation of fish to low oxygen concentrations. Diurnal and other fluctuations of dissolved oxygen concentration in fish habitats can be great and fairly rapid. However, fish which are entirely unaccustomed to moderately low oxygen concentrations are not often exposed abruptly to critical concentrations which they cannot avoid even temporarily. The gain and loss of the resistance of trout with acclimatization to different oxygen concentrations were not rapid in Shepard's experiments at temperatures near 9° C. but perhaps are accelerated at higher temperatures. There is need for further intensive investigation of the magnitudes, rates, and persistence of changes of tolerance produced by acclimatization.

Experiments in which young salmonids were held for a day or longer at uniform, low dissolved oxygen concentrations in continually renewed, flowing, partially deoxygenated water, which had been deoxygenated naturally (well water) or artificially (by means of nitrogen bubbled through the water), have been reported by Townsend and Earnest (1940), Townsend and Cheyne (1944), Graham (1949), Davison (1954) and Shepard (1955). The ability of some trout and salmon to tolerate dissolved oxygen concentrations near, or even well below, 2 p.p.m. at moderate temperatures has been demonstrated by such tests. Davison succeeded in keeping all of a group of ten young silver salmon, *Oncorhynchus kisutch*, alive for 4 weeks in water (partially deoxygenated with nitrogen) with a dissolved oxygen concentration averaging 2.0 p.p.m. (range 1.8 to 2.4 p.p.m.) at a temperature of 18° C. However, the fish were not active, consumed little food, and lost weight during the experiment; while salmon held in water with 3 p.p.m. dissolved oxygen fed well and gained weight. Results of experiments in progress at Oregon State College indicate that, under some conditions, concentrations well above 3 p.p.m. may be necessary for rapid growth and even for long survival of younger silver salmon, as well as for normal development and successful hatching of silver salmon eggs.

The resistance of warm-water fishes to low dissolved oxygen concentrations has not been experimentally investigated as thoroughly as that of the cold-water salmonids. There are many indications, however, that the salmonids are among the freshwater fishes which are most sensitive to reduction of the dissolved oxygen content of their medium. Comparative tests, such as those of Burdick *et al.* (1954), have shown warm-water forms to be more resistant. The following general conclusions based on the available data, which cannot be considered in detail here, are presented by way of summary of information gleaned from the voluminous literature on the dissolved oxygen requirements of freshwater fishes. Dissolved oxygen concentrations well above 3 p.p.m. apparently can be quite rapidly fatal, at fairly high temperatures and under otherwise unfavorable conditions, to susceptible fishes which are not accustomed to low oxygen concentrations, and also to relatively resistant species under exceptionally adverse conditions. However, mortality of fishes at concentrations near and above 3 p.p.m. is not to be expected ordinarily, even when the low concentrations persist for long periods. Concentrations in the neighborhood of 2 p.p.m. may be often critical for sensitive forms. Yet, at moderately low temperatures and under otherwise favorable conditions, concentrations near and below 1 p.p.m. evidently can be tolerated for long periods not only by the most resistant species, but also by more susceptible fishes which have been thoroughly acclimatized to low dissolved oxygen concentrations.

It should be remembered that persistent nonlethal deficiency of dissolved oxygen undoubtedly can adversely influence the activities of fishes and have serious detrimental effects on fish populations in their natural environments. These effects and pertinent studies already have been considered in the introductory discussion of water quality requirements in general.

### B. Oxygen and Nitrogen Excess (Supersaturation)

Injury to fish by abnormally high concentrations of dissolved oxygen has been reported, but Wiebe and McGavock (1932) have shown that young trout, various centrarchids, and minnows can withstand very long exposure to concentrations ranging from 20 to 34 p.p.m., or from two to more than three times the corresponding air-saturation values, without apparent harm. Supersaturation of water with atmospheric gases (chiefly nitrogen and oxygen) can, however, cause fatal "gas-bubble disease" of fish when the total pressure of the dissolved gases (i.e. the sum of their individual tensions) greatly exceeds the hydrostatic pressure, including the pressure of the atmosphere. Such supersaturation can result from mechanical injection of air into water under pressure greater than atmospheric pressure followed by release of the high pressure, from warming of cold air-saturated water out of contact with air, and probably also from intense photosynthetic activity of planktonic algae. It can be corrected by vigorous aeration, which removes the excess gases. The body fluids of fish held in the supersaturated water evidently soon become also supersaturated with atmospheric gases. Bubbles then form within the tissues, just as they may form also in the external medium, especially when it is agitated, collecting on any surface such as the exterior of the fish, or on glass wool. Very different, involved, and curious explanations of gas-bubble disease have been advanced repeatedly, but apparently they are unnecessary. The disease has been ascribed usually to nitrogen bubbles and sometimes to oxygen bubbles, but Engelhorn (1943) found that the composition of the bubbles corresponded initially to that of the gas mixture with which the external medium had been charged under pressure. The percentage of oxygen decreased with time, while that of inert nitrogen increased. Gas-bubble disease can be produced by creating a partial vacuum over air-saturated water in which fish are held, thus reducing the hydrostatic pressure. Mere bubbling of pure nitrogen or oxygen through water, which drives out one gas and substitutes another without increasing the total gas tension, evidently cannot cause the disease.

## VI. SUSPENDED SOLIDS (TURBIDITY)

Many freshwater fishes occur in very turbid waters. Some species may avoid such waters, and fishes may not thrive in them, because of various indirectly harmful effects on their environments of the turbidity and of the deposition of solids. Developing eggs of fish, as well as fish-food organisms, may be smothered by deposits of silt (Smith, 1940). However, direct injury to fully developed fish by nontoxic suspended matter apparently has been demonstrated convincingly only in tests with concentrations which are most uncommon in both natural and polluted waters.

Wallen (1951) reported results of numerous experiments designed to determine the direct effect of montmorillonite clay turbidity on warm-water fishes of 16 species. He cites many other references. Usually he observed no behavioral reactions of the experimental fish until the turbidity values neared 20,000 p.p.m. (as $SiO_2$), and some species reacted only to much higher turbidities. With the exception of only a few individuals of 2 species, the death of which may have been due largely to causes other than turbidity, the fish died only when the turbidity values were well above 50,000 p.p.m. According to Wallen, most of the experimental fishes "enduring exposure to more than 100,000 p.p.m. of turbidity for a week or longer, but these same fishes finally died at turbidities of 175,000 to 225,000 p.p.m. Lethal turbidities caused the death of fish within 15 minutes to 2 hours after the onset of exposure. Fishes that succumbed to turbidity had opercular cavities and gill filaments clogged with silty clay particles from the water."

Clogging or blanketing of the gills of fish with matter suspended in water, such as soil particles, fibers, and precipitates, is not unusual when fish are exposed to various toxic solutions containing the suspended material. Injury to the gills by harmful dissolved substances probably results in the inability of the fish to keep their gills clean. The clogging of the gills may possibly contribute sometimes to the injurious action of the toxic media, without being the primary cause of injury, which it may seem to be. Interference with gill functions ascribable to chemically inert suspended matter as the primary cause or factor apparently can occur only when the concentrations of suspended matter are exceedingly high, so that it is of little practical importance.

## VII. MISCELLANEOUS TOXIC SUBSTANCES

### A. Variety, Sources, and Literature

Any one of a great number of toxic substances can render water unsuitable for fish. Some of these are of natural origin, deriving from mineral deposits or from vegetable matter, such as certain algae, leaves, and barks. Toxic products of bacterial decomposition of vegetation and of domestic and industrial organic wastes discharged into watercourses include ammonium compounds and inorganic sulfides. Industrial wastes, such as effluents from gas and coke plants, oil refineries, metal-finishing and electroplating plants, pulp mills, and various chemical factories, mine drainage, and oil field brines, all of which are common water pollutants, can be highly toxic to fish. They may contain acids or alkalis, salts of various metals, cyanides, phenols, and other toxicants of endless variety. Extremely poisonous insecticides, herbicides, and algicides can cause catastrophic mortality of fishes when they are purposely added to water, or are washed into watercourses from land by rains.

Many studies in fish toxicology have been undertaken in connection with water pollution investigations and the development of chemical methods for the control of aquatic pests or nuisances in fish habitats. Others have been purely pharmacological or physiological studies, planned without regard to possible application of the results in connection with practical water-quality problems. These studies have contributed to the understanding of the effects of water pollution on fish, even though many of the chemicals tested are not important contaminants of water. The use of fishes, such as goldfish, as test subjects in biological assays of the potency or concentration of drug preparations and solutions of chemicals has been repeatedly recommended. The early work of Powers (1917) is of historical interest in this regard.

The toxicity of thousands of chemicals to fish having been studied in various ways and for different reasons by many investigators, the literature on fish toxicology is voluminous and widely scattered and cannot be easily summarized. In the absence of sufficiently complete and accurate compendia, students of the subject must rely heavily on original sources of needed data. The early treatise on fish toxicology of Steinmann (1928), and the general discourses and summaries of literature by Redeke (1927), Helfer (1936), Ellis (1937), Cole (1941), Southgate (1948), and Harnisch (1951) are useful reference works. Ellis (1937) and, more recently, the California State Water Pollution Control Board (1952) have compiled summaries of published data on the toxicity to fish of a large number of water pollutants. Though not complete, these are very useful guides to the pertinent literature; however, the data reported should be verified by consulting original references. The first two parts of a comprehensive, critical review of literature on the toxicity of industrial wastes and their components to fish by Doudoroff and Katz (1950, 1953) have been published. These treat of the toxicity of alkalis, acids, inorganic gases, and the metals as salts. Much of the following discussion is based on information contained in these two reviews, wherein additional data and references to source literature can be found.

### B. Mode of Action and Penetration of Toxicants

The injurious action of many substances which can be fatal to fish when dissolved in their medium is believed to be external or very superficial, absorption into the blood and other internal tissues being apparently negligible. Damage to the delicate tissues of the gills and impairment of their respiratory and excretory functions undoubtedly can be fatal. Ellis (1937) presented evidence that the circulation of blood in the gills (through gill capillaries), as well as the flow of water over the external cells of the gill filaments, can be interfered with by an external film of coagulated mucus, which coats and clogs the gills and immobilizes the gill filaments. Westfall (1945) noted that the susceptibility of goldfish to substances which are believed to cause death by anoxia through formation of such a coagulation film (sulfuric acid, lead nitrate) increased when the dissolved oxygen concentration of experimental solutions was reduced.

The effects of external, superficial intracellular, and truly internal action of lethal agents on fish are not always readily distinguishable. All the injurious effects ascribable to chemical action upon living or nonliving matter constituting these organisms (not their external environment) are considered herein as toxic effects.

Substances which cause internal injury enter through gill and other exposed surfaces or may be absorbed from the gastrointestinal tract after ingestion of contaminated water. Marine fishes normally ingest much sea water in connection with their osmoregulation, and ingestion of some water by freshwater fishes has also been demonstrated. The speedy intoxication of freshwater fishes immersed in sufficiently concentrated solutions of most of the internal poisons of outstanding importance as water pollutants indicates rapid absorption of these substances through external surfaces. There is evidence, however, that these surfaces are not penetrated readily by many substances, and particularly by various ions. The effects of some compounds which quickly cause superficial damage are clearly referable to their ions.

The internal action of most poisons on fish tissues probably does not differ fundamentally from their action on the tissues of warm-blooded and other terrestrial vertebrates. However, concentrations which are harmless in water ingested by any animal in limited quantities obviously can be far greater than the maximum concentrations tolerated by fish in water continually bathing their gills. Furthermore, environmental factors which can have no influence on the activity of a poison taken internally can greatly influence its external activity and the rate of its penetration through external surfaces. Ionic equilibria in solutions in which fish are immersed are important in this connection, because of selective permeability of the external membranes.[2]

It is noteworthy also that compounds generally thought of as being virtually insoluble in water may be quite soluble enough to render water highly toxic to fish. Substances said to be virtually undissociated in aqueous solutions, having numerically very small dissociation constants (e.g. mercuric chloride, some complex metallocyanide ions, etc.), actually can be largely or almost completely dissociated in exceedingly dilute yet toxic solutions. The high degree of dissociation is ascribable to the very low concentrations of the solutions, and sometimes also to partial removal of one of the products of the dissociation by combination with available hydrogen or hydroxyl ions.

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[2] It should be noted that the terminology of water chemistry is often inconsistent and misleading. So-called "free carbon dioxide" in water includes $CO_2$ present as dissolved gas and as undissociated $H_2CO_3$, but not as carbonate and bicarbonate ions, while "free ammonia" includes ammonium ions, as well as undissociated $NH_4OH$, considered as $NH_3$. The expression "cyanide ion concentration" (also "free cyanide concentration") is commonly used in referring to the total concentration of cyanide ion and molecular HCN (expressed as $CN^-$), although the term "hydrogen ion concentration" has to do with the actual concentration of ions only. This confused terminology can be very troublesome when it is employed, as it often must be, in toxicological literature. The distinction between the molecular acids or bases and their respective ions cannot be disregarded, in view of differences of their physiological activity and penetrative properties.

General principles introduced above will be illustrated in discussing the toxicity of some important types of water pollutants.

### C. Antagonism and Synergism

The antagonism of metal cations, some of which markedly counteract the toxicity of others in mixed salt solutions, has already been mentioned. Not infrequently, some constituents of mixtures of chemicals are synergistic, one contributing to, or intensifying, the injurious action of another (Bandt, 1946; Southgate, 1948). The terms antagonism and synergism are strictly applicable only to the action of combined substances or ions which do not lose their identity in the mixtures through chemical reaction. However, these terms are often used somewhat loosely when referring to interactions of uncertain nature, which may be simply chemical reactions the products of which are less toxic or more toxic than the reacting substances.

Examples of synergism and antagonism are given in connection with discussions of the toxicity to fish of some typical water pollutants selected for special consideration. They show that estimates of the degree of toxicity of complex solutions (such as industrial effluents and most polluted waters) can be grossly erroneous if based on determinations of the toxicity of each component in the absence of antagonistic or synergistic substances present in the mixtures. It is noteworthy that the dissolved minerals present in most fresh waters can greatly influence the toxicity of a large number of different pollutants, through antagonism or chemical reaction. Suspended matter, such as silt, can have a similar effect, through adsorption of dissolved toxicants.

### D. Toxicity Bioassays

Since the degree of toxicity to fish of complex industrial wastes and other pollutants in various receiving waters is usually unpredictable, experimental determination by an appropriate biological assay method is frequently necessary. Satisfactory control of waste disposal often cannot be achieved without such toxicity evaluation. Many bioassay methods have been described and used by investigators in the past. The need for some standardization of procedure, designed to improve the reliability and the comparability of toxicity test results, has been increasingly apparent. Progress toward such standardization has been made in recent years. In the United States, methods for the evaluation of acute toxicity of wastes and other water pollutants to fish recommended by a committee in the Federation of Sewage and Industrial Wastes Associations (Doudoroff *et al.*, 1951) have been widely adopted. They are intended chiefly for frequent routine application in connection with waste disposal control, but serve also as a basic pattern for somewhat more elaborate and refined toxicological research methods. A joint effort to achieve some uniformity of method has been made also by some students of fish toxicology in Europe (Wuhrmann *et al.*, 1953).

Truly chronic toxicity of water pollutants to fish has been studied very infrequently, and even acute toxicity tests often have not been sufficiently prolonged to yield results of any practical value. Some toxicants, such as zinc salts, which can be fatal within a day or two in solutions of very low concentration are tolerated for 1 or 2 hours in comparable solutions with concentrations more than 100 times as great. Fishes used as experimental animals have been often unusually hardy species. For these and other reasons, the toxicity of some important water pollutants has been slighted by many investigators.

In addition to the nature of the water employed (i.e. its total alkalinity, pH, hardness, turbidity, etc.), the species of fish selected, and the duration of the tests, many other considerations are important in connection with the planning of toxicity tests and the appraisal of published results. The choice and uniformity of experimental temperatures are of outstanding importance. Rates of intoxication usually (but not always) increase markedly with rising temperature, and they may vary also with the thermal history of the test animals. While the total number of fish used in each test must be adequate, the number and weight of fish held in a limited volume of experimental medium (test solution) which is not renewed frequently or continually must be restricted. Otherwise, the concentrations of toxicant in very dilute solutions may decline rapidly during tests, because of its accumulation in the tissues of the fish, metabolic destruction, or combination with mucus. Furthermore, dissolved oxygen must be maintained at an adequate level, without speedy removal of any volatile constituents of the experimental medium by inordinate artificial aeration of the medium or otherwise. The depth of vessels used, the size of experimental fish, their previous history, health, and acclimation to laboratory conditions, and the recency of their feeding, all are important considerations.

## E. Strong Alkalis and Acids

The toxicity of the common strong alkalis, sodium, potassium, and calcium hydroxides, and that of the common strong acids, sulfuric, hydrochloric, and nitric, can be referred usually to hydroxyl ions and hydrogen ions, respectively, the action of which may be mostly external. Fatal concentrations of $CO_2$ can be liberated by the strong acids in waters with a very high bicarbonate content. When free $CO_2$ is not present in harmful concentrations, solutions of the named compounds with pH above 5.0 and not above 9.0 apparently are harmless to fully developed freshwater fishes. The pH then appears to be an adequate index of dangerous contamination of water with one of these substances. The maximum amounts of strong acid or alkali that can be added to equal volumes of unequally buffered natural waters without rendering them toxic to fish obviously can differ greatly. Sodium carbonate solutions appear to be at least as toxic as NaOH solutions with corresponding pH, or slightly more toxic.

### F. Ammonia and Ammonium Compounds

As little as 2 to 7 p.p.m. of ammonia, or ammonium hydroxide as $NH_3$, has been repeatedly reported to be fatal to fishes when added to fresh waters. Solutions of this weak base can be very toxic when the pH is well below 9.0. The toxicity evidently is not referable directly to hydroxyl ions. However, it can be markedly reduced or eliminated by neutralization of the base with acids. Solutions of ammonium salts in naturally buffered alkaline waters can be highly toxic also, but the toxicity decreases with decreasing pH, as well as with decreasing ammonium content.

Wuhrmann, Zehender, and Woker (1947) found that the toxicity of ammonium sulfate solutions of varying concentrations, and with pH adjusted to different values between 7.0 and 9.5, was determined by the computed concentration of nonionic ammonia, or molecular ammonium base. When this concentration was between 2 and 3 p.p.m. as $NH_3$ (in solutions with total ammonium concentrations of 10.1, 38.4, and 167 p.p.m., and pH 8.84, 8.16, and 7.52, respectively) their experimental fish (*Squalius cephalus*) lost equilibrium in 170 to 330 minutes. When it was increased to 16–24 p.p.m. as $NH_3$, either by increasing the ammonium content of the solutions without change of pH, or by merely raising the pH, the effective exposure time was reduced to 26 or 27 minutes. It can be concluded that ammonium ions in the external medium are nearly or entirely harmless. The base presumably acts internally.

### G. Weak Acids and Inorganic Sulfides

Phosphoric acid and some moderately weak organic acids (e.g., lactic, citric, tartaric), like the strong mineral acids, have not proved directly injurious to freshwater fishes in solutions with pH above 5.0. However, the toxicity of more acid solutions sometimes may be referable to undissociated acid molecules, rather than hydrogen ions. Westfall (1945) ascribed the lethality of lactic acid solutions to internal effects (acidemia), following penetration of the acid into the blood stream.

A number of weak acids, like carbonic acid or dissolved free $CO_2$, can impart pronounced toxicity to water without lowering the pH to 5.0. This group of acids includes very weak and toxic hydrosulfuric and hydrocyanic acids, much less toxic boric acid, and apparently also much stronger acetic and propionic acids. The toxicity must be referable to undissociated molecules or to anions or salts of these acids. The salts of many are known to be relatively harmless in alkaline solutions. Thus, neither the pH nor the anion concentration by itself is a reliable index of dangerous pollution with one of these acids.

Longwell and Pentelow (1938) noted a marked decrease of the toxicity of sodium sulfide solutions to trout when the pH was raised. Undissociated $H_2S$ (produced by hydrolysis of $Na_2S$) evidently is more toxic than the anions of this acid. However, the inorganic sulfides have proved fatal to sensitive fishes such as trout at concentrations between 0.5 and 1.0 p.p.m. as S even in neutral and somewhat alkaline solutions. Much of the sulfur can exist as $H_2S$ in such solutions, the dissociation constant of hydrosulfuric acid being sufficiently small.

### H. Cyanides and Metal–Cyanide Complexes

Hydrocyanic acid, a virulent respiratory poison which acts internally, ionizes very little in waters which are not markedly alkaline. The alkali metal cyanides in dilute solutions with pH below 9 are mostly converted, through hydrolysis, into volatile molecular HCN. Wuhrmann and Woker (1948) found that HCN molecules are more injurious to fish than cyanide ions. *Squalius cephalus* lived considerably longer in highly alkaline cyanide solutions than in less alkaline solutions.

Karsten (1934) and Herbert and Merkens (1952) showed that trout may be overcome within 1 to 6 days by 0.05–0.07 p.p.m. of cyanide (as $CN^-$) in flowing, continually renewed KCN solutions. Karsten found that 0.02 p.p.m. as $CN^-$ did not kill brook trout in 27 days, however; and extrapolation of a curve plotted by Herbert and Merkens indicates that this concentration may not be fatal to rainbow trout (at 17.5° C. and pH 7.4–8.0) within their normal life span even if there is no higher threshold concentration. The susceptibility of fish to cyanides is markedly influenced by temperature, thermal history, and dissolved oxygen concentration.

There is no evidence that any of the metal-cyanide complex ions, such as $Zn(CN)_4^{--}$ and $Ni(CN)_4^{--}$, are directly harmful to fish. However, the zinc–cyanide and cadmium–cyanide complexes (with reported dissociation constants near $10^{-17}$) must dissociate almost completely in solutions of complex cyanides when the concentrations are less than 0.5 p.p.m. as $CN^-$; the cyanide ions so liberated combining with available hydrogen ions to form molecular HCN. The highly toxic products of this decomposition of the complexes in dilute solutions (HCN and metallic ions) apparently are somewhat synergistic. The author's experimental results (Doudoroff, 1956) show that complexation of cyanide with zinc and cadmium certainly effects no significant reduction of toxicity to fish.

Complexation of cyanide with metals which form relatively stable cyanide complexes, such as nickel, copper, and iron, can result in great reduction of the toxicity of solutions of cyanides and salts of heavy metals (e.g. extremely toxic cupric salts) when they are combined. However, when combined with nickel to form the nickelocyanide complex, 1.0 p.p.m. of cyanide (as $CN^-$) at pH 6.5 proved more toxic to minnows (*Pimephales promelas*) than 1000 p.p.m. at pH 8.0. At the lower pH the toxicity of solutions of the complex cyanide approaches that of NaCN solutions with equivalent cyanide content. Taking into consideration the ionic equilibrium equations for the dissociation of the nickelocyanide ion and also of hydrocyanic acid, it was concluded that when the hydrogen ion concentration of $Na_2Ni(CN)_4$ solutions is halved in raising the pH from 7.5 to 7.8, the concentration of nickelocyanide ion must be increased about sixteen-fold in order to maintain the initial concentration of molecular HCN. The concentration of the complex cyanide fatal to half the minnows used as test animals within 24, 48, or 96 hours increased about ten-fold or thirteen-fold with rise of pH from 7.5 to 7.8. This observation confirms the logical supposition that the toxicity of the solutions is determined mostly, if not entirely, by the concentration of molecular HCN.

While nickel and copper sulfates apparently form cyanide complexes readily when added to extremely dilute, faintly alkaline cyanide solutions, ferrous sulfate evidently does not. Burdick and Lipschuetz (1950) reported that harmless potassium ferrocyanide and ferricyanide solutions became highly toxic to fish when exposed to direct sunlight, because of liberation of cyanide through photodecomposition of the otherwise stable complexes.

The toxicity of solutions of unstable and very toxic lactonitrile has been attributed to hydrocyanic acid, but that of solutions of other, relatively stable nitriles, such as acrylonitrile, which is considerably less toxic than lactonitrile (Daugherty and Garrett, 1951), may not be referable to HCN. Detailed investigation of the toxic action of these compounds is needed.

### I. The Metals as Salts

Silver, mercury, copper, lead, cadmium, zinc, aluminum, nickel, and chromium have been classed as metals of relatively high toxicity to fish (Doudoroff and Katz, 1953). Salts of the most toxic metals, particularly silver, mercury, and copper, have been reliably reported harmful to fish in some fresh waters at concentrations near 0.01 p.p.m. of metal (0.004–0.02 p.p.m.). Nickel and chromium, which apparently are considerably less toxic than the other metals named (and perhaps should be classed as metals of intermediate toxicity, with cobalt, possibly iron, and others) proved injurious at concentrations near 1 p.p.m. (Jones, 1939). Moderately toxic cobaltous, manganous, barium, lithium, and potassium ions have been classed as metal cations of relatively low toxicity to fish, together with the ions of magnesium, calcium, strontium and sodium, which certainly are among the least toxic of the metals.

Water which comes in contact with elemental metals may be rendered thereby highly toxic to fish, especially when it is very soft water with much free $CO_2$ and low pH. The potency of the metals in this respect depends on the readiness with which soluble salts, such as bicarbonates, are formed, as well as the relative toxicity of the salts. Thus, copper and zinc can be extremely injurious where lead (also nickel, iron) would be harmless.

Moderate concentrations of metal salts which are harmless to fish in sea water and highly mineralized fresh waters can be extremely injurious in very soft fresh waters. The toxicity of sodium, magnesium, and potassium ions, and of highly toxic heavy metal cations such as lead, zinc, and cupric ions, is markedly counteracted by calcium ions and by other antagonistic metal cations. Calcium ion concentrations usually found in natural fresh waters (10 to 50 p.p.m.) can be very effective. Calcium and sodium ions are mutually antagonistic. On the other hand, copper with zinc (also copper with cadmium, and zinc with nickel) are strongly synergistic. Thus, 0.025 p.p.m. of copper with 1.0 p.p.m. of zinc in mixed solutions of the sulfates prepared with soft water proved more rapidly fatal to minnows than 0.2 p.p.m. of copper or 8.0 p.p.m. of zinc alone.

The lethal action of the salts of many metals is not yet well understood. Internal penetration of some has been demonstrated. The lethality of heavy metals (lead, copper, zinc, mercury) has been ascribed, however, to coagulation of mucus (i.e. precipitation of insoluble metal-protein compounds) on gill surfaces and damage to gill tissues, and to consequent respiratory failure. It has been reported that in contaminated sea water, fish may absorb considerable amounts of heavy metals, such as copper, before being evidently harmed. Yet, relatively minute concentrations of these metals in fresh waters can be rapidly fatal when there has been virtually no absorption of metal, but only superficial accumulation (Carpenter, 1927).

Jones (1938) found that calcium salts not only counteracted the toxicity of lead and zinc salts, but also prevented precipitation or coagulation of mucus by the heavy metals on the gills of fish and in filtered eel slime. The action of lead on the mucus was not reversed by the addition of calcium after precipitation had occurred; and fish affected by lead nitrate recovered no more rapidly in water with added calcium than in untreated soft tap water.

Unbuffered solutions of salts of many metals (e.g. aluminum, iron, manganese, chromium) can be highly acid, because of hydrolysis. Precipitation of insoluble compounds of the metals, such as hydroxides and basic salts, occurs often when these salts, and some other metal salts also, are added to natural waters. The concentration of active metal remaining in solution (e.g. cupric ion) may vary inversely with pH and directly with the concentration of free $CO_2$, derived from decomposed bicarbonates. The $CO_2$ escapes when the solutions are aerated, causing further precipitation of metal. The toxicity of the solutions usually is clearly referable to dissolved metal. However, the lethality of waters contaminated with iron salts has been ascribed to hydrogen ions (Jones, 1939), to precipitated iron hydroxide settling on the gills (Bandt, 1938; Sanborn, 1945), and to probably semi-colloidal basic iron salts penetrating gill tissues (Laroze, 1950), as well as to iron in solution. The influence of iron on fish, which has been judged both great and negligible, evidently needs much additional investigation.

Jones (1935) showed that at the same molar concentration the sulfate of a heavy metal (e.g. copper, zinc) can be much less toxic to fish than the corresponding chloride and nitrate. He noted and discussed a relation between the toxicity of comparable, rapidly fatal solutions of the different salts and the electrical conductivity. However, the influence of harmless anions deriving from metal salts added to ordinary natural waters upon the limits of tolerable concentration of the metal cations still needs thorough investigation. Its practical importance in connection with the lethality of the most toxic metals has not been firmly established (Doudoroff and Katz, 1953).

The toxicity of salts such as chromates and permanganates obviously is not generally referable to metal cations. Hexavalent chromium (as chromates or dichromates) is not so acutely toxic to fish as trivalent chromium (as chromic salts). Permanganates, on the other hand, are far more toxic than manganous salts, 2 to 4 p.p.m. as Mn being rapidly fatal.

### J. Other Toxic Water Pollutants

Free chlorine and chloramines, which are commonly present in treated municipal water supplies, have been reported to be decidedly toxic to susceptible freshwater fishes at concentrations equivalent to about 0.05 to 0.3 p.p.m. of available chlorine. The harmful action of these powerful oxidizing agents may be superficial. Cyanogen chloride, which is formed on chlorination of thiocyanate solutions such as sewage containing gas plant wastes, also is extremely toxic, concentrations near 0.1 p.p.m. having proved fatal to trout.

Various phenolic compounds present in many complex industrial effluents are very toxic to fish, but their toxicity varies. The phenol content of polluted waters and sewage, determined often in connection with their sanitary chemical examination, is not a measure of the concentration of ordinary phenol (carbolic acid) alone, and therefore has no definite toxicological significance. Estimates of the relative toxicity of various combinations of toxicants present in most phenolic wastes certainly cannot be based on the available data on the toxicity of phenol, which have been reviewed recently by Wuhrmann and Woker (1950).

Some of the organic insecticides are among the most toxic to fish of all known substances. The chlorinated camphene known as toxaphene has been shown to be fatal to hardy goldfish at concentrations near 0.005 p.p.m. (Doudoroff, Katz, and Tarzwell, 1953). Other synthetic organic insecticides now widely used in the control of pests, and rotenone, which is commonly employed as a fish poison for the eradication of undesirable fish populations, also are extremely toxic.

A number of synthetic detergents have been shown to be acutely toxic to various freshwater fishes at concentrations well below 10 p.p.m. Unlike ordinary soaps, many, though not all, of these compounds are as toxic in hard waters as in very soft waters (LeClerc and Devlaminck, 1952). Degens *et al.* (1950) found that the resistance of fish to various anionic and nonionic detergents increased with gradual acclimatization. Fishes which were killed rapidly by 5 p.p.m. of these detergents were said to have tolerated concentrations as high as 9 to 36 p.p.m. after acclimatization at progressively increasing nonlethal concentrations.

There is no point in enumerating any more of the vast array of toxic organic and inorganic chemicals which may occur in polluted waters since the known effects and the individual toxicity of each obviously cannot be considered within the space of this chapter.

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## CHAPTER X

# PHYSIOLOGICAL GENETICS OF FISHES

MYRON GORDON

Page
- I. Introduction . . . . . . . . . . . . . . . . . . . . . . 432
- II. Sex Determination . . . . . . . . . . . . . . . . . . . 433
  - A. Dual Genetic Mechanisms in Fishes . . . . . . . . . . . 433
    - 1. The XX Female, XY Male Mechanism . . . . . . . . . 434
    - 2. Crossing-over of Sex Chromosomes . . . . . . . . . . 435
    - 3. The WY Female, YY Male Mechanism . . . . . . . . . 435
    - 4. Dual Mechanisms in Natural Populations . . . . . . . 436
  - B. Stability of the Sex Chromosomes . . . . . . . . . . . 436
    - 1. All-Male Broods . . . . . . . . . . . . . . . . . 436
    - 2. Broods Containing Three Females to One Male . . . . . 437
  - C. Evolution of the Dual Mechanism . . . . . . . . . . . . 437
  - D. Incompatibility of Sex Chromosomes in Hybrid Fishes . . . . 440
    - 1. First Generation Hybrids . . . . . . . . . . . . . . 440
    - 2. Second Generation Hybrids . . . . . . . . . . . . . 441
  - E. Indeterminate Methods of Sexual Differentiation . . . . . 442
    - 1. Hybrids in Natural Populations . . . . . . . . . . . 442
    - 2. Hybrids in Aquarium-Reared Fishes . . . . . . . . . . 443
    - 3. Parthenogenesis . . . . . . . . . . . . . . . . . 445
    - 4. Transformation of Functional Females to Functional Males . . 446
- III. Effects of Pigment Cell Genes . . . . . . . . . . . . . . 447
  - A. Deleterious Effects of Recessive Genes . . . . . . . . . 447
  - B. Deleterious Effects of Dominant Genes . . . . . . . . . 448
  - C. Control of Chromogens . . . . . . . . . . . . . . . . 449
  - D. Interaction with Specific Modifying Genes . . . . . . . . 450
  - E. Interaction between Two Pigment Cell Genes . . . . . . . 452
- IV. Genes Influencing Atypical Pigment Cell Growth . . . . . . . 453
  - A. Frequency and Comparative Pathology . . . . . . . . . . 453
  - B. Genic Imbalance for Melanomas in Hybrids . . . . . . . . 455
  - C. Multiple Factors in the Abnormal Growth of Macromelanophores . 458
  - D. Interracial Matings and Their Effect upon the Expressivity of Pigment Cell Genes . . . . . . . . . . . . . . . . . . . . 458
  - E. Erythromelanomas and Spacial Relationship of Color Genes . . 462
  - F. Albino Gene for Amelanotic Melanomas . . . . . . . . . . 464
- V. Eye Characteristics . . . . . . . . . . . . . . . . . . 464
  - A. Cataracts Traced in Five Generations of Platyfish . . . . . 464
  - B. The Genetic Basis for the Development of Ocular Tumors . . . 464
  - C. Flicker Responses . . . . . . . . . . . . . . . . . . 465
  - D. Blindness of Cave Forms . . . . . . . . . . . . . . . 466
- VI. Meristic Traits . . . . . . . . . . . . . . . . . . . . 468
  - A. Frequencies of Vertebrae . . . . . . . . . . . . . . . 468

B. Abnormal Vertebrae . . . . . . . . . . . . . . . . . 469
C. Frequencies of Fin Rays . . . . . . . . . . . . . . . . 470
D. The Shape of Some Meristic Characters . . . . . . . . . . 473
VII. Transplantation, Resistance, Serology . . . . . . . . . . . . . 473
A. Transplantation . . . . . . . . . . . . . . . . . . 473
B. Temperature Tolerance . . . . . . . . . . . . . . . . 475
C. Resistance to Infections, Rate of Growth, and Maturity . . . . . 475
D. Effects of Ultraviolet Radiation . . . . . . . . . . . . . 477
E. Serological Reactions . . . . . . . . . . . . . . . . 478
VIII. Susceptibility to Thyroid Tumors . . . . . . . . . . . . . . 478
A. Frequencies and Relationship to Low Iodine Concentration of Water . 478
B. Influence of Heredity . . . . . . . . . . . . . . . . 479
1. Thyroid Tumors in the Montezuma Swordtails . . . . . . . 479
a. Progressive growth . . . . . . . . . . . . . . . 483
b. Variation in sublines . . . . . . . . . . . . . . 484
2. Thyroid Tumors in the Kidneys of the Common Platyfish . . . 484
a. One subline resistant, one susceptible . . . . . . . . . 485
IX. Isolating Mechanisms and Speciation . . . . . . . . . . . . . 486
A. Psychological or Mating Behavior Patterns . . . . . . . . . 486
B. Embryological Rates of Development . . . . . . . . . . . 490
X. Summary . . . . . . . . . . . . . . . . . . . . . 492
A. Evidence of Genetic Influences . . . . . . . . . . . . . 492
B. Need for Inbred Stocks . . . . . . . . . . . . . . . 493
References . . . . . . . . . . . . . . . . . . . . . 493

## I. INTRODUCTION

In the introduction to his pioneering book "Physiological Genetics," Richard Goldschmidt (1938) said that the problems of heredity that are concerned simply with the *mechanism* of gene transmission from parents to their progeny are generally referred to the study of *genetics* proper. But there is another aspect to the problems of heredity which is concerned with an understanding of how individual genes, interacting genes, or a constellation of genes act in controlling the development of the adult organism. Since these developmental processes are tied up with gene action, Goldschmidt suggested that its subject matter may be termed *physiology of heredity* and the science devoted to its solution, *physiological genetics.*

More specialized problems of physiological genetics have been treated by Beadle (1945) in his review of Biochemical Genetics, by Haldane (1954) in "The Biochemistry of Genetics," and by Wagner and Mitchell (1955) in "Genetics and Metabolism." While these publications discuss no experimental data gathered from studies of fishes, they contain much information, particularly of physiological processes under genetic influence, that are likely to apply to the lower vertebrates.

Most of the growing literature on the genetics of fishes concerns physiological processes, but unfortunately in most instances these processes have not been studied intensively. The review which follows attempts to state those physiological aspects in fishes for which genetic influences are known, even though they are known incompletely.

## II. SEX DETERMINATION

In his book, "The Biology of Fishes," Kyle (1926) looked back to the last century and accepted the theory of the physiological distinction between the sexes as expressed by Geddes and Thompson (1889): "The female is physiologically more passive, the male more active." Kyle alleged that while Mendel's laws of heredity had led to fruitful investigations in the study of sex determination in other organisms, they had not been taken up to any extent in the study of fishes. He claimed that "the natural conditions are all against such exact investigations, and where artificial cultures have been undertaken, as in Johs. Schmidt's (1919, 1920) experiments, . . . the results have been rather against the Mendelian theory."

Schmidt (1920) showed that a color trait, a black spot in the dorsal fin of one stock of colorful guppies, *Lebistes reticulatus*, was inherited "one-sidedly," that is, from father to son, father to son, etc.; the females which were without bright colors or markings apparently did not contribute to the color inheritance of their sons. This kind of male sex-linked and male sex-limited inheritance was a novelty in 1919. The results, when announced, were certainly not in conformity with the usual mode of Mendelian inheritance. And yet, Schmidt offered an adequate explanation which suggested that the black spot of the male guppy may be referred to a factor that is carried by a sex chromosome that also determines maleness. This was soon confirmed independently by Aida (1921) in the medaka, *Oryzias latipes*, and by Schmidt's successor, Winge (1922, 1923) in the guppy. Kyle (1926), however, did not refer to these papers, nor to the much earlier clear demonstrations of *simple* Mendelian inheritance in the medaka by Ishikawa (1913), Toyama (1916), and Ishihara (1917).

### A. Dual Genetic Mechanisms in Fishes

If Kyle's textbook on ichthyology revealed a lack of appreciation of Winge's initial work on the guppy, most of the authors of current genetic texts have revealed that they have not adequately analyzed Winge's later (1932, 1934) data. This is unfortunate because the information available on genetic mechanisms for sex determination in laboratory and natural populations of fishes is far more extensive and critical than in any other vertebrate group. For example, it will be recalled (White, 1954) that mammals have a genetic mechanism in which the female is homogametic

(XX) and the male is heterogametic (XY), while birds have the opposite type in which the female is heterogametic (WY) and the male homogametic (YY). In fishes both systems, the XX-XY and WY-YY, are represented. In the guppy and in the medaka, the genetic mechanism is XX female, XY male, like that in mammals. In the platyfish (*Xiphophorus maculatus*) from four separate Mexican rivers, the mechanism is also XX female, XY male; but in the geographical races of the *same* platyfish species from British Honduras, the mechanism is WY female, YY male (Gordon, 1951a). This dual system in a single species is unique. It is probable that the mode of evolution of the dual genetic mechanisms for sex determination in vertebrate animals may eventually be solved by careful study of sex linkage in the poeciliid fishes.

### 1. The XX Female, XY Male Mechanism

The inheritance of the color character, a black spot in the dorsal fin known as the *maculatus* of the guppy, first studied by Schmidt, and then by Winge, illustrates a type of sex linkage which contributes to the conclusion that *Lebistes* has a type of genetic sex determination in which the female is XX, the male XY. In the formulas which follow, the symbols for the various sex chromosomes are capitalized and placed within parentheses; the genes that they carry are italicized. Thus when a dominant *maculatus* (*Ma*) male guppy is mated to a recessive (+) female the results may be expressed as follows:

| | Females | Males |
|---|---|---|
| $P_1$ | (X) + (X) + | (X) + (Y)*Ma* |
| $F_1$ | (X) + (X) + | (X) + (Y)*Ma* |
| $F_2$, $F_3$, etc. | (X) + (X) + | (X) + (Y)*Ma* |

This type of "one-sided" (male to male) inheritance was found also by Aida (1921, 1930) for the *red* (*R*) gene in the medaka and by Gordon (1947a) for the *spotted-dorsal* (*Sd*) gene in the members of the Rio Jamapa populations of the platyfish.

Additional evidence for the XX female, XY male type of sex linkage in the guppy, medaka, and the Mexican platyfish was obtained from matings that showed "criss-cross" inheritance. This involves a gene that is transmitted from a father exclusively to his daughters, or from a mother to her sons. When a nonspotted (+) female Mexican platyfish was mated to a *spotted* (*Sp*) male, they produced spotted daughters and nonspotted sons (Gordon, 1947a):

| | Females | Males |
|---|---|---|
| $P_1$ | (X) + (X) + | (X)*Sp* (Y) + |
| $F_1$ | (X) + (X)*Sp* | (X) + (Y) + |

In the guppy, criss-cross inheritance may appear to be nonexistent because the female ordinarily is incapable of expressing the color characteristics in accordance with its genotype. For example, a *lutius* (*Lu*) male guppy mated to a recessive female will produce $F_1$ sons and daughters neither of which show the appropriate trait. The explanation is that most color traits are not only sex-linked, but their appearance is sex-limited to the male guppy. This may be demonstrated by carrying the mating through the second generation. In the genetic series of matings given below, note that female guppies of the first generation have the genotype (X) + (X)*Lu*, yet they are without color:

| | | | Females | | Males | |
|---|---|---|---|---|---|---|
| $P_1$ | (colorless) | | (X) + (X) + | | (X)*Lu*(Y) + | *lutius* |
| $F_1$ | (colorless) | | (X) + (X)*Lu* | | (X) + (Y) + | *non-lutius* |
| $F_2$ | (colorless) | 1. | (X) + (X) + | 3. | (X)*Lu*(Y) + | *lutius* |
| | (colorless) | 2. | (X) + (X)*Lu* | 4. | (X) + (Y) + | *non-lutius* |

When females are treated with testosterone, they are capable of expressing the type of coloration that is in accordance with their genotype.

### 2. CROSSING-OVER OF SEX CHROMOSOMES

Crossing-over of the sex chromosomes in fishes was first clearly demonstrated by Winge (1923) in the guppy, and some of the exceptional types recorded by Aida (1921) were explainable on the same basis. Later Fraser and Gordon (1929) and Gordon (1947a) confirmed the possibility of crossing-over of the sex chromosomes in the platyfish. The following example is from Aida (1930) who mated a red male medaka with a white female:

| | | Females | Males |
|---|---|---|---|
| $P_1$ | | (X) + (X) + | (X)*R* (Y) + |
| $F_1$ | (expected) | (X) + (X)*R* | (X) + (Y) + |
| $F_1$ | (crossover) | | (X) + (Y)*R* |

### 3. THE WY FEMALE, YY MALE MECHANISM

The opposing type of sex-determining mechanism in fishes in which females are heterozygous WY, the males homozygous YY was discovered in the domesticated stocks of the platyfish by Bellamy (1922) and shortly confirmed independently by Gordon (1927) and Kosswig (1928). This may be demonstrated by the criss-cross type of inheritance as follows, in which a *spotted* (*Sp*) female is mated to a plain (+) male:

| | Females | Males |
|---|---|---|
| $P_1$ | (W) + (Y)*Sp* | (Y) + (Y) + |
| $F_1$ | (W) + (Y) + | (Y) + (Y)*Sp* |

Fraser and Gordon (1929) detected crossing-over of the sex chromosomes by genetic techniques in the WY *spotted* (*Sp*) platyfish females as follows:

| | Females | Males |
|---|---|---|
| $P_1$ | (W) + (Y)*Sp* | (Y) + (Y) + |
| $F_1$ (expected) | (W) + (Y) + | (Y) + (Y)*Sp* |
| $F_1$ (crossover) | (W)*Sp*(Y) + | |

The test for the exceptional crossover (W)*Sp*(Y) + was as follows:

| | Females | Males |
|---|---|---|
| $P_1$ | (W)*Sp*(Y) + | (Y) + (Y) + |
| $F_1$ | (W)*Sp*(Y) + | (Y) + (Y) + |

This mating also demonstrated a new kind of inheritance, that is, mother to daughter, since that was the way the *spotted* gene was transmitted subsequently.

Some geneticists have suggested that a female when heterogametic for sex chromosomes be indicated as XY and a male when homogametic be indicated as XX. This would lead to confusion when representatives of both mechanisms are intermated (Gordon, 1952). One could not know, taken out of context, if XX (or XY) referred to a male or a female.

### 4. Dual Mechanisms in Natural Populations

Gordon (1951c) announced that the WY-YY mechanism was not only found in the domesticated stocks of platyfish but also in the members of natural populations. These were from the Belize River in British Honduras (and in the adjacent New River, unpublished data). The availability of Mexican platyfish, some of which have the XX female, XY male mechanism, while other members of the same species from British Honduras have the WY female, YY male system, has made possible intermatings between them. These matings between members of the different geographical races have produced results indicating that the Y chromosome in both races carries genetic factors for maleness. The X chromosome carries factors for femaleness and the W has a similar effect only it is somewhat more powerful than X in the feminizing reaction.

## B. Stability of the Sex Chromosomes

### 1. All-Male Broods

The following racial crosses will illustrate the preciseness of reaction in platyfish in response to the various sex chromosomes involved. Our advantage here is that the various sex chromosomes may be "tagged" by genes that produce specific color markings in female platyfish as well as in males:

| | | |
|---|---|---|
| P$_1$ | Stripe-sided Female from Río Jamapa<br>(X)*Sr*(X)*Sr* | Spotted-sided and Spotted dorsal Male from Belize River<br>(Y)*Sp*(Y)*Sd* |
| F$_1$ | Females (0%) | Males (100%)<br>50% (X)*Sr*(Y)*Sd*, Spotted-dorsal<br>50% (X)*Sr*(Y)*Sp*, Spotted-sides |

If one of the F$_1$ striped and spotted Jamapa-Belize hybrids, (X)*Sr*-(Y)*Sp*, is mated back to a pure Jamapa female, (X)*Sr*(X)*Sr*, note the "one-sided" (male to male) inheritance of the *spotted* gene, *Sp*, among these backcross hybrids:

| | Females | Males |
|---|---|---|
| P$_1$ | (X)*Sr*(X)*Sr* | (X)*Sr*(Y)*Sp* |
| F$_1$ | (X)*Sr*(X)*Sr* | (X)*Sr*(Y)*Sp* |

### 2. Broods Containing Three Females to One Male

Reciprocal matings between the same geographical races from Mexico and British Honduras reveal additional evidence of the stability of reactions of the various sex chromosomes:

| | Female from Belize River | Male from Río Jamapa |
|---|---|---|
| P$_1$ | (W) + (Y)*Sp* | (X)*Sr*(Y)*Sd* |
| F$_1$ | Females (50%) | Males (50%) |
| | 1. (W) + (X)*Sr* | 3. (X)*Sr*(Y)*Sp* |
| | 2. (W) + (Y)*Sd* | 4. (Y)*Sd*(Y)*Sp* |

If an F$_1$ stripe-sided female (number 1) (W) + (X)*Sr* is mated to a stripe-sided, spotted male (number 3) (X)*Sr*(Y)*Sp*, three F$_2$ females are produced to *one* male (sex, indeed, is a Mendelian character):

| | Females (75%) | Males (25%) |
|---|---|---|
| F$_2$ | 1. (W) + (X)*Sr* | 4. (X)*Sr*(Y)*Sp* |
| | 2. (W) + (Y)*Sp* | |
| | 3. (X)*Sr*(X)*Sr* | |

## C. Evolution of the Dual Mechanism

Winge (1932, 1934) suggested a method for the evolution of the dual sex-determining mechanism in fishes that was based upon his discovery of a continuous series of sex reversals of XX female guppies into males. The discovery of XX males (and XY females) led him to two assumptions: (1) genes in the sex chromosomes may be neutralized by opposing sex genes in the autosomes; (2) powerful sex genes may become concentrated in a single pair of autosomes in such a way that an autosomal pair of chromosomes is capable of functioning subsequently like a pair of sex chromosomes.

Actually, there is no factual evidence to support the second assumption that the exceptional XY female guppies are heterogametic and the XX males are homogametic on the basis of a single pair of sex-determining autosomes. Since reviews have accepted this assumption as fact it is worth-while to re-examine and re-evaluate the original data upon which this assumption is based. After Winge (1932) detected an exceptional XX male, he used it for an unusual series of backcrossings as follows: the original XX male was mated to one of its XX daughters. He obtained all females as expected. Then he mated the same XX male to one of its XX granddaughters and again obtained all females. He then mated the same XX male to one of its XX great granddaughters. From the last mating Winge obtained another exceptional XX male which he mated, this time, to its mother. The outcome of the XX son to its XX mother mating was reported as follows: "males and females segregated apparently in a 1:1 ratio," but no actual figures were given. However, in other matings involving XX males and XX females Winge (1932, 1934) and Winge and Ditlevsen (1948) found the following frequencies of the sexes:

| Year | No. of Matings | Males | Females | Ratio |
|---|---|---|---|---|
| 1932 | 5 | 37 | 34 | 1.1:1.0 |
| 1934 | 24 | 84 | 344 | 1:4.1 |
| 1948 | . . | 26 | 851 | 1:32.7 |
| | Total | 147 | 1229 | 1:8.3 |

Thus it may be seen that XX males mated to XX females do not usually produce equal numbers of sons and daughters. Therefore to assume that the sex-determining mechanism has been transferred from the sex chromosomes to a pair of autosomes is out of the question. Working under this assumption Winge (1934) attempted, but failed, to link a specific pair of autosomes carrying the *zebrinus* gene in *Lebistes* to the "new sex-determining chromosomes." Later, Winge and Ditlevsen (1948) again failed in a similar attempt when they studied other autosomes.

The fact that the exceptional *Lebistes* XX males and XY females are precariously balanced with regard to the sum total of their sex genes has been indicated by Winge and Ditlevsen (1948). They reported that when the sex-reversed XY females and XX males from different lines are outcrossed, the normal sex-determining mechanism, XX female, XY male, is re-established immediately in an aquarium population as follows:

| | Female | Male |
|---|---|---|
| $P_1$ Sex-Reversed Parents | (X) + (Y)*Ma* | (X)*Li*(X)*Li* |
| $F_1$ (Reconstituted) | 32 Females<br>(X) + (X)*Li* | 35 Males<br>(X)*Li*(Y)*Ma* |

In the process of genetic segregation, the effective female influencing autosomal sex genes in the $P_1$ XX males and the effective male influencing autosomal genes in the $P_1$ XY females apparently cancel each other out. As a result of the outcross, the balance between all genes for sex is restored and the sex-determining mechanism is again dependent upon the usual combinations of X or Y chromosomes, so that the $F_1$ males are again XY and the females are XX.

Despite uncritical statements to the contrary, the sex-determining mechanism in some fish is stable and the sex of the individual is determined at conception. In the platyfish, for example, environmental influences play a minor role in altering the genetic sex of the individual. This is subject to test. The mating of Rio Jamapa XX female platyfish with a YY male from Belize River produced 183 offspring, all of which were male. The sex of these 183 $F_1$ young was determined in a natural way, that is, they were grown to maturity and all of them developed typical gonopodia and other indisputable characters of the male sex at about 6 months to 1 year. The Rio Jamapa $P_1$ female platyfish that produced all male young continued to have more young. Forty of its immature $F_1$ were fixed, some when 1 day old, others when 28 days old, 73 days old, and so on. None of these $F_1$ showed any definite secondary sexual characters at the time when they were fixed. From a histological analysis of their gonads, Chavin and Gordon (1951) found that all of them had incipient or definitive testes, none had any ovarian elements. Thus they were males as far back in their development as histological methods were able to reveal.

Occasionally the usual sex chromosome mechanism of XX for femaleness in the Rio Jamapa platyfish is overridden. Gordon (1947a) found an XX male in the Río Jamapa stock which was later studied histologically by Gordon and Aronowitz (1951). They discovered that its gonad, although hypertrophied, was functional for a brief period. When the exceptional Río Jamapa XX male was mated to a normal XX female of a similar stock, all their offspring were normal females, XX, as one would expect on theory. This and other known sex-reversed individuals are important in establishing the type of sex-determination in the members of a particular population but they have little bearing upon the evolution of the system itself.

The conclusions reached from the genetic analysis of the sex-determining mechanisms in the guppy, the medaka, and the platyfish are as follows: (1) Female and male sex-determining genes are probably distributed over a great many of the autosomes with superior sex genes in the sex chromosomes. (2) The superior sex genes in the sex chromosomes may, in exceptional individuals, be overridden by many opposing autosomal genes each with small effects upon sex determination. (3) It is unlikely, nor has it been demonstrated, that a pair, any *one pair*, of autosomes has ever taken over the role of the sex chromosomes. (4) It is extremely doubtful that exceptional XX male and XY female guppies which are occasionally detected in the laboratory occur in many populations that live under natural conditions. (5) XX males and XY females are unstable and could not be maintained in natural populations where members live in a state of panmixia. (6) Female heterogamety (WY-YY) in the platyfish of British Honduras and male heterogamety (XX-XY) in the platyfish of Mexico constitute stable sex-determining mechanisms in each natural population. (7) Both mechanisms probably have evolved by processes of natural selection, the complete details of which are as yet unknown.

## D. Incompatability of Sex Chromosomes in Hybrid Fishes

### 1. First Generation Hybrids

The degree of sterility in the platyfish–swordtail hybrids (genus *Xiphophorus*) depends primarily upon the type of mating, the genotype of the hybrid, and particularly the presence or absence of the Y chromosome of the platyfish. For example, Gordon (1948) found if the wild platyfish from the Río Jamapa is the female parent and the wild swordtail from the Río Papaloapan is the male parent, the sex ratio among their $F_1$ hybrids is approximately as follows: 90% female, 10% male. This mating can be expressed genetically by indicating the various sex chromosomes "marked" by appropriate genes: *Sp* represents irregular macromelanophore spotting along the sides of the platyfish, and *Sd* represents macromelanophore spotting on the dorsal fin. The female platyfish sex chromosomes may be identified by XX. Comparable chromosomes of the male swordtail are indicated arbitrarily by X′X′ although sex linkage in this species is as yet unknown:

$P_1$ Platyfish Female, (X)*Sp*(X)*Sd* × Swordtail Male, (X′)+(X′)+
$F_1$ (X)*Sp*(X′)+: spotted hybrids; 45% females, 5% males
(X)*Sd*(X′)+: spotted dorsal hybrids; 45% females, 5% males

Tavolga (1949) showed that 33 (X)*Sp*(X′)+ platyfish-swordtail female hybrids tested for fertility revealed only 2 which were completely sterile; and 3 which were of low productivity producing aberrant broods, each of which contained over 30% anomalous embryos. Of 34 (X)*Sd*(X′) + female hybrids, 15 were completely sterile, 11 possessed aberrant broods, and 8 produced normal broods. Four out of 5 (X)*Sd*(X′) + male hybrids and 4 out of 5 (X)*Sp*(X′) + male hybrids were fertile when tested with various virgin platyfish, swordtails, or hybrids.

If the wild swordtail from Rio Papaloapan is the female parent (indicated arbitrarily as X′X′) and the wild, stripe-sided, spotted-dorsal platyfish from the Rio Jamapa is the male (XY), the sex ratios are quite different, as follows:

$P_1$ Swordtail Female, (X′) + (X′) + × Platyfish Male, (X)*Sr*(Y)*Sd*
$F_1$ (X)*Sr*(X′) + : 50% stripe-sided hybrid females; mostly fertile;
(no males)
(Y)*Sd*(X′) + : 2% spotted-dorsal hybrid males; fertile
3% spotted-dorsal hybrid females; fertile
45% spotted-dorsal hybrids; sterile

The first 10 (X)*Sr*(X′) + hybrid females tested proved to be fertile, and the assumption was made that the rest were probably also fertile.

Two (Y)*Sd*(X′) + hybrid males proved to be fertile. It was surprising that three hybrid females presumably of the same genetic constitution (Y)*Sd*(X′) + were also fertile, at least partially so, for they produced a few living young. None of these females approached the high degree of fecundity of the (X)*Sd*(X′) + female hybrids. Most of the (Y)*Sd*(X′) + hybrids, amounting to approximately 45% of the entire brood, had no visible gonads, and they attained a body size greater than that of the other hybrids.

From these details it appears that the X chromosome of the platyfish is fairly compatible with its theoretical counterpart, the X′ chromosome of the swordtail, but the platyfish Y chromosome, for the most part, is incompatible with the X′. The degree of compatibility of the platyfish X chromosome with the swordtail X′ chromosome varies with the type of gene the platyfish X carries. *Sr* and *Sp* genes on the X in combination with X′ produce fertile hybrids, but the *Sd* on the X in combination with X′ carries with it some sterility. When *Sd* is carried on the Y chromosome, most of the platyfish–swordtail hybrids are sterile.

### 2. SECOND GENERATION HYBRIDS

The $F_2$ hybrids were obtained by mating an (X)*Sd*(X′) + female to an (X)*Sp*(X′) + male. Each of the $F_2$ hybrids had one of three sex chromosome combinations as indicated in Table I. The sex chromosome combination was determined phenotypically by examining the macromelanophore pattern of the fish. At the same time the condition of the gonad was determined for each of the $F_2$ hybrids and then related to the sex chromosome combination.

TABLE I

SEXUALITY IN $F_2$ PLATYFISH-SWORDTAIL HYBRIDS[a]

| Sex Chromosome Combinations | Gonadal Development | | | | | Total |
|---|---|---|---|---|---|---|
| | Wanting | Immature | | Mature | | |
| | | Female | Male | Female | Male | |
| 1. $(X') + (X') +$ | 1 | 5 | 0 | 36 | 44 | 86 |
| 2a. $(X)Sp(X') +$ | 26 | 22 | 1 | 28 | 6 | 83 |
| 2b. $(X)Sd(X') +$ | 17 | 20 | 2 | 42 | 7 | 88 |
| 3. $(X)Sp(X)Sd$ | 28 | 31 | 0 | 22 | 0 | 81 |
| | 72 | 78 | 3 | 128 | 57 | 338 |

[a] From Berg and Gordon (1953).

## E. Indeterminate Methods of Sexual Differentiation

### 1. HYBRIDS IN NATURAL POPULATIONS

In a pond formed by damming a tributary of the Pocantico River, which flows into the Hudson River in New York State, Bailey and Lagler (1938) discovered three species of sunfish (some native, some introduced) which, owing to the limited spawning areas, produced a considerable number of hybrids. The species involved were the green sunfish, *Lepomis cyanellus*, the common sunfish, *L. gibbosus*, and the bluegill, *L. macrochirus*. Intense crowding and competition for nesting sites was held responsible for the large hybrid population, which amounted to 11.7% of the total. Since hybridization was indiscriminate among the three species, the authors suggested that the barrier to hybridization was neither a differential choice of mates nor a differential incompatibility of their germ cells. But Clark, Aronson, and Gordon (1954) thought that there must have been some germ cell incompatibility in these hybrids because the sex ratio was four or more males to one female. This deviation from the normal 1:1 sex ratio might indicate that sunfishes have a type of genetic (or chromosomal) mechanism for sex determination in which the females are heterogametic, the males homogametic. This would be in accordance with Haldane's rule (1922), which states that "when in the first generation hybrids between two species, one sex is absent, rare, or sterile, that sex is always the heterogametic sex."

In this connection, ichthyologists have described many apparent hybrids that are allegedly found in wild populations of fishes. Some are referred to as hybrids between distinct species, others between genera. The descriptions of the alleged hybrids and suspected parents are stated in precise meristic terms and the pigmentary patterns are elaborately delineated. And yet only a few observers have indicated the histological state of gonads, one of the most important criteria in evaluating the authenticity of any hybrid.

### 2. Hybrids in Aquarium-Reared Fishes

Berg and Gordon (1953) have classified histologically the various gonadal failures in male and female platyfish–swordtail hybrids which may be used for purpose of general comparison (Table II). In the absence of experimental verification of hybridization, such as Hubbs and Hubbs (1933) have made for some hybrid sunfish, a histological description of the gonads in all presumed natural hybrids is highly desirable. And in experimentally verified hybrids, the study of the gonads would be helpful in adding to our knowledge of the biology of natural populations of fishes.

Recently cytological analysis of the gonads was made of eight Anatolian oviparous cyprinodonts of the genera *Kosswigichthys*, *Aphanius*, and *Anatolichthys* and their hybrids by Öztan (1954). Many of the abnormalities described above in the platyfish–swordtail hybrids were also recorded for these Turkish killifish hybrids. Öztan found that all the hybrid males were sterile while most females had intersexual traits; some females were partially fertile. The variability of sexuality in the females was related to the parental species. She reported that although the male hybrids were sterile and some had extremely reduced testes, they were able to express their normal secondary sexual characters with reference to color and behavior. This coincides with some of the findings of Clark, Aronson, and Gordon (1954) for platyfish–swordtail hybrids.

That Anatolian killifish hybrid males are sterile and females partly fertile might indicate that the parental fish have the XY-male–XX-female type of sex determination. This could not be confirmed cytologically for no sex chromosomes were identifiable, although the total number of chromosomes for the various species was either 44, 46, or 48. Many viviparous cyprinodont fish have chromosome numbers that fall into this range according to Friedman and Gordon (1934), Wickbom (1941) and Matthey (1949).

In the absence of observable heterochromosomes or sex-linked characters that may serve as chromosome markers, it is difficult to establish the type of genetic mechanism for sex determination. When in the offspring of a given species 1:1 sex ratios are observed, the heterogametic

## TABLE II
### HISTOLOGICAL ANALYSIS OF TESTICULAR STRUCTURE IN PLATYFISH–SWORDTAIL HYBRIDS

| Condition of Testes | | $(PX)^2$ [a] | PX-X [b] | P-PX [c] | Total |
|---|---|---|---|---|---|
| *Normal* | | 10 | 15 | 0 | 25 |
| *Minor abnormalities:* | | | | | |
| a | excess colloid | 0 | 5 | 4 | 9 |
| b | unencapsulated sperm in ducts, few | 2 | 1 | 0 | 3 |
| c | early stages of spermatogenesis, scarce | 1 | 3 | 3 | 7 |
| d | interstitial tissue, slightly abnormal | 0 | 0 | 3 | 3 |
| *Major abnormalities:* | | | | | |
| b′[*] | unencapsulated sperm filling ducts | 7 | 3 | 0 | 10 |
| d′ | interstitial tissue, abnormal | 0 | 0 | 3 | 3 |
| e′ | sperm in ducts, scarce | 0 | 3 | 0 | 3 |
| f′ | immature, no sperm | 3 | 8 | 2 | 13 |
| g′ | teratoid testis | 2 | 2 | 0 | 4 |
| a′ + b | excess colloid and unencapsulated sperm in ducts | 11 | 4 | 1 | 16 |
| b′ + c | unencapsulated sperm and scarcity of early spermatogenic stages | 4 | 1 | 0 | 5 |
| a′ + f | excess colloid and few sperm in ducts | 6 | 5 | 1 | 12 |
| a′ + d | excess colloid and abnormal interstitial tissue | 4 | 0 | 0 | 4 |
| d′ + e | interstitial tissue abnormal and gonad immature | 0 | 0 | 1 | 1 |
| a′ + b′ + c | excess colloid, unencapsulated sperm and scarcity of early spermatogonial stages | 9 | 2 | 2 | 13 |
| a′ + d′ + f | excess colloid, abnormal interstitial tissue and a scarcity of sperm in ducts | 0 | 0 | 3 | 3 |
| Total number of testes examined | | 60 | 52 | 20 | 132 |
| Number of testes with minor abnormalities | | 3 | 9 | 10 | 22 |
| Number of testes with major abnormalities | | 47 | 28 | 10 | 85 |

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[a] $(PX)^2$ = P, platyfish; X, swordtail; $F_2$ hybrid from a mating of an $F_1$, PX female and an $F_1$, PX male.

[b] PX-X = P, platyfish, X, swordtail backcross hybrid from a mating of a PX female and a swordtail male.

[c] P-PX = P, platyfish, X, swordtail, backcross hybrid from a mating of a platyfish female and a PX male.

[*] ′ = The symbol ′ applied to a lower case letter indicates a major abnormality.

type is attributed to one sex and the homogametic is assigned to the other, but this is unsatisfactory for it is arbitrary. When the sex-determining mechanism is known in one species and hybridization is possible between it and an unknown but related species, sometimes the results indicate the type of sex determination in the second species. For example, Kosswig (1935a) mated a *maculatus* platyfish male of the domesticated strain, known to be homozygous, to a *variatus* platyfish female in which the sex-determining mechanism was unknown. Only male $F_1$ hybrids appeared and Kosswig concluded that in *variatus* the female is homogametic and by extension the male is probably heterogametic. Kosswig (1936) obtained similar results in testing *xiphidium* females. These conclusions were confirmed by Gordon (1944) who utilized sex-linked genes in establishing the fact that the females of *variatus* and of *xiphidium* are homogametic while the males are heterogametic.

Kosswig's (1954) modified generalized theory of sex determination in fishes, which is based upon environmental as well as genetic factors, it is claimed, applies to xiphophorin fishes and their hybrids. The theory had been applied also by Breider (1935) to three species of *Limia* (*nigrofasciata*, *caudomaculata*, and *vittata*) and their hybrids; to three species of the paradise fish *Macropodus* (*opercularis*, *concolor*, and *chinensis*) and their hybrids, originally described by Schwier (1939); and to the fighting fish, *Betta splendens*, described by Umrath (1939). Attempts to explain our data obtained from study of *Xiphophorus helleri* and their various hybrids on the basis of Kosswig's theory have been unsuccessful. The sex-determining mechanism in the swordtails *montezumae* as well as *helleri* remains an unsolved problem chiefly because no sex-linked characters have as yet been discovered in these species. The results obtained by means of hybridization have been inconsistent with every theory suggested. The complexity of the problem is also discussed by Hämmerling (1942–1944).

### 3. Parthenogenesis

Several species of mollies, *Mollienesia*, have featured prominently in studies devoted to the analysis of apparent parthenogenesis in fishes. Hubbs and Hubbs (1932) announced that they crossed *M. sphenops* to *M. latipinna* and obtained hybrids that resembled a species previously known as *M. formosa*. They also announced that female hybrids (*M. formosa*) mated to males of either parental species, or to males of *Lebistes*, *Limia*, or *Gambusia*, produced female offspring only, all of which were similar to their "*formosa*" mother; none were intermediates. Meyer (1938) believed that the chromosomes of the male entered the egg at fertilization but were later inactivated. Recently, Hubbs (1955) has reiterated his conclusion concerning apparent parthenogenesis in "*formosa*" regarding it as a "good species" of hybrid origin, having a definite natural range. Since these conclusions have been published without support of any quantitative data, they cannot be evaluated objectively. In *Lebistes*, Spurway (1953) claimed parthenogenetic births for nine young. Later, in a letter, she said that sectioned ovaries of other virgin mothers revealed some testicular tissues. This may account for P. B. Medawar's cryptic remark (1957) that virgin guppies may be self-fertilizing. But some proof is needed. The alleged fatherless fishes should be tested by skin or fin transplants (see page 473).

### 4. Transformation of Functional Females to Functional Males

It has often been cited that fishes have a labile mechanism for sex determination. This generalized, oversimplified and unwarranted conclusion has been derived in a large measure from Essenberg's (1926) alleged examples of complete and functional sex reversal in the swordtail, *Xiphophorus helleri*. Essenberg's claim rests on only two swordtails which allegedly were *functional females* first and then were *functional males*, but the evidence for these radical changes is subject to criticism owing to lack of supporting data. From the numerous citations referring to these alleged functional sex-reversed swordtails the reader gets the erroneous impression that the phenomenon is common in this species, whereas even the alleged instances are extremely rare. During the past quarter of a century not another instance of spontaneous, complete and functional sex reversal has been reported and verified; yet during this long period many species of viviparous cyprinodont fishes, including swordtails, have been laboratory bred and studied by the thousands. Essenberg (1923) regarded the swordtail as a protogynous species, claiming many of its young pass through a *female-like stage of gonadal development* before becoming functional males. In a contrary and far more substantial report Friess (1933) found that sexuality in *X. helleri* is quite similar to that in the platyfish, *X. maculatus*. The gonads of post-natal platyfish did not change according to Wolf (1931).

From studies of the sea bass, *Centropristes*, living under natural conditions, data have been presented by Lavenda (1949) which indicate that the smaller members of the population are females while the larger ones are males. Ovotestes are found in the intermediates. These data are interpreted to mean that functional sex reversal is a natural process in this species and in other species where conditions are somewhat similar. But until the complete history of their sexuality is known over the individual sea bass' complete life cycle, it would be premature to accept the conclusion that functional sex reversal actually takes place

## III. EFFECTS OF PIGMENT CELL GENES

### A. Deleterious Effects of Recessive Genes

Color differences between fish of the same species are often regarded as being rather superficial, but some of the genes affecting color patterns are definitely associated with a number of major physiological disturbances. In the swordtails (*Xiphophorus helleri*), for example, Gordon (1942) demonstrated that color genes for golden and albinism are responsible in some manner, as yet unknown, for the death of the light-colored fish during their late embryonic development.

The ancestral, olive-gray coloring of the wild swordtail is a mosaic pattern of two major kinds of tiny pigment cells, the blacks (micromelanophores) and the yellow (xanthophores). By a mutational change in its hereditary constitution, the wild olive-colored swordtail lost most of the black cells in its skin but it retained its yellow ones. Gordon (1938) showed that the golden coloration of the swordtail (*gg*) was recessive to the gray (*GG*) and was typically Mendelian.

The albino swordtail with its light body coloring and pink eyes was discovered about 1934 in aquaria containing ordinary, black-eyed, olive-gray swordtails. The albino mutation (*aa*) brought about the almost complete elimination of the black pigment normally present in the skin, in the retina of the eyes, and in all other areas of the body. Curiously, the mutant appeared almost simultaneously in American and in European aquaria. Kosswig (1935b) first showed that albinism in the swordtail is a recessive hereditary trait.

When Gordon (1941) mated a golden swordtail with an albino he obtained in the first generation the wild, fully pigmented swordtail. He explained this phenomenon of reversion to the wild type, saying that each parental color variety, the golden and albino, brought to its $F_1$ offspring one of the essential dominant factors which the other lacked, as follows:

| | | | | |
|---|---|---|---|---|
| Golden | × | Albino | = | Wild, Olive-gray |
| *gg AA* | × | *GG aa* | = | *Gg Aa* |

When 4 pairs of $F_1$ wild-colored swordtails were mated brother to sister (*Gg Aa* x *Gg Aa*), the results in the second generation showed fewer albinos and golden than one might expect on the basis of the expected 9:3:4 ratio.

In order to determine whether the embryos died in their later stages of development, several of the same gravid, wild gray $F_1$ swordtail female parents (*Gg Aa*) that had been mated to *Gg Aa* males were killed and dissected. Their $F_2$ embryos were sorted out according to their phenotypes and counted. They were found in the ratio of 9 gray to 3 yellow to 4 albino in an almost perfect result. Apparently many of the albino and golden embryos die just short of their full term. The exact nature of the cause of death has not been discovered. Haskins and Haskins (1948) have reported almost identical results with the gray, albino, and golden guppies, *Lebistes reticulatus*.

The golden mutation in the platyfish, *Xiphophorus maculatus*, is also a partial lethal (Gordon, 1927), as is the albino gene in the paradise fish, *Macropodus* (Goodrich and Smith, 1937; Schwier, 1943). Comparable genes determining golden coloration do not seem to affect the genetic ratios in the medaka, *Oryzias latipes* (Ishihara, 1917; Aida, 1921) or in the Siamese fighting fish, *Betta splendens* (Goodrich and Mercer, 1934; Eberhardt, 1941). Rosenthal (1951) called the recessive gene for lordosis in guppies a semi-lethal and Winge and Ditlevsen (1938) claimed that a recessive gene linked to the dominant *maculatus* gene is lethal.

### B. Deleterious Effects of Dominant Genes

In the platyfish, Kosswig (1938) described the partial lethal effect of the dominant gene *Fu* (fuligonosus) for the sooty black effect produced by many macromelanophores. Apparently *Fu* has a somewhat lethal effect in the heterozygous state and even more in the homozygous state. Unfortunately his data are not definitive but the following mating, one of three made by Kosswig, will indicate the nature of the results: (+ represents the recessive sex-linked genes; *Dr* represents the dominant sex-linked gene for red dorsal):

$P_1$ Fuligonosus Female, $(W)+(Y)Fu$ × Fuligonosus Male, $(Y)Fu(Y)Dr$

$$\begin{array}{lllll} F_1 & \text{Females} & & \text{Males} & \\ & 27\ (W)+(Y)Fu & (29) & 45\left\{\begin{array}{l}(Y)Fu(Y)Fu\\ \\ (Y)Fu(Y)Dr\end{array}\right\} & (58) \\ & 45\ (W)+(Y)Dr & (29) & & \end{array}$$

From a total 117 $F_1$ fish only the 45 non-fuligonosus females are in excess of the expected frequencies (which are given within the parentheses). The discrepancies are probably much greater than the estimates based on a total of 117 fish. It is likely that 45 (W) + (Y)*Dr* (dorsal red females) actually represent one-fourth of the theoretical total number of the original progeny. If this were so, then there should have been 45 fuligonosus females rather than 27, and 90 fuligonosus males rather than 45.

Kosswig declared that *Fu* males develop melanotic overgrowths on the gonopodium, the modified anal fin which functions as a copulatory organ. This abnormality prevents the males from transferring their spermatophores to the females. Thus the *Fu* gene has a deleterious effect on two counts. The growth may have a lethal effect in some members of the brood and it may prevent some adult males from effective insemination of females. Öktay (1954) says that the aberrant frequencies of about 2 *Fu* to 1+ in the $F_2$ broods, instead of 3:1, are brought about by differential incompatibility of *Fu* sperm and *Fu* ova. Data by Gordon and Baker (1955) indicate, on the other hand, that the *Fu* gene when homozygous is lethal; *FuFu* fish do not live beyond 35 days of age. Ermin (1953) revealed that the *Fu* gene interacts with the *R* gene for red coloring and produces erythromelanomas in many platyfish carrying *FuR* genes. The precise genetics of this and the previous situation need further study but there is no question that the effects described are genetically induced.

In his analysis of the principal genes in the Russian carp, *Cyprinus carpio*, Kirpichnikov (1937) found a variety that had a complete or almost complete absence of scales. This condition was inherited through the dominant autosomal gene *N*, *nude*. When this gene was in a heterozygous state *Nn* it was viable; when homozygous *NN* it was lethal. In other scaleless strains of German carp, the hereditary picture must be different because these fish are viable. Later Kirpichnikov (1945) indicated that the *N* gene in the heterozygous phase upsets normal embryogenesis resulting partly in abnormal numbers and arrangement of pharyngeal teeth and lowered resistance to poor environmental conditions of food and temperature.

### C. Control of Chromogens

The problem of determining the nature of the interrelations between the gene and the visible inherited character was approached by Goodrich (1927) by studying the embryonic and later stages of the development of three color phases in the Japanese medaka, *Oryzias latipes*. He recognized that a single gene may influence many characters and that a single character may be the end product of the interaction of many genes. The problem is undoubtedly complex, involving the interplay of developmental processes such as embryonic segregation and induction, cell differentiation and migration, and the influence of hormones and exogenous factors.

Aida (1921) and others had shown that the wild brown medaka and its variegated and its yellow colored mutants are genetically determined by a series of autosomal alleles: *B*, *B′*, and *b*. *B* is dominant over *B′* and *B′* is dominant over *b*. Goodrich indicated that the three color phases could be distinguished histologically on the basis of the frequency and location of their chromatophores, particularly the black cells or melanophores. A superficial view of the skins of the varieties revealed that the darkest fish contained the largest number of melanophores, the lightest one the fewest, and the variegated one had many melanophores in some areas and few in others. When, however, the three mutants were treated with adrenaline, a more accurate pigmentary analysis of their colors was possible. Pigment cells which were previously overlooked because their contained melanin granules were too scattered to produce a visible effect were detected when the pigment was withdrawn to the center of the cell. Goodrich discovered that the *b* gene was not responsible for the elimination of melanophores but the gene reduced the amount of melanin the melanophore could synthesize.

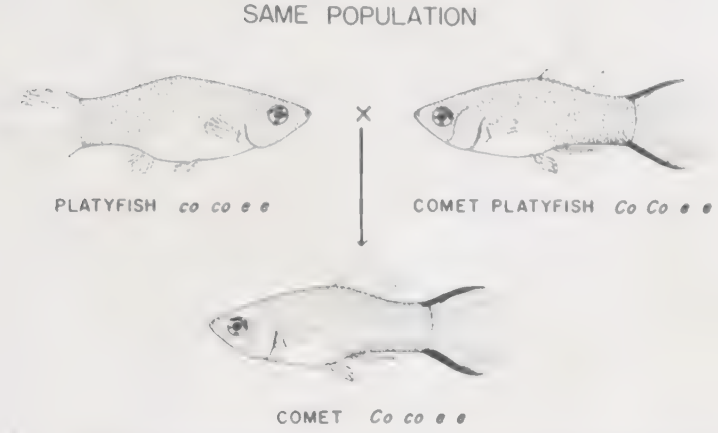
A further analysis of the physiological genetics of the three color phases of the medaka was made by Goodrich (1933) who utilized the "dopa" technique which is capable of determining the presence of the oxidase of dihydroxyphenylalanine. He found that "dopase" was present in the pale melanophores and from this he concluded that these light cells are deficient in their ability to produce sufficient chromogen: gene *B* induces formation of a full complement of normally pigmented melanophores; gene *B′* induces approximately half normal melanophores and half lightly colored melanophores; gene *b* produces all lightly colored black pigment cells.

### D. Interaction with Specific Modifying Genes

A gene, even though it be dominant, may not have any immediate visible expression of its own. A gene of this nature was detected by Gordon (1938) by its effect when in the presence of a second hereditary factor. The following example, taken from Gordon (1946a) will show that the interaction of the two genes produces a special effect. The comet platyfish is characterized by one black line along the upper margin and another black line on the lower margin of the caudal fin. This pattern may be referred to the comet gene (*Co*) which behaves as a simple Mendelian dominant factor with reference to the absence of that pattern (*co*), as follows (Fig. 1):

$P_1$ Comet (*CoCo*) × Non-Comet (*coco*)
$F_1$ Comet (*Coco*) × Comet (*Coco*)
$F_2$ 3 Comet: 1 *CoCo*, 2 *Coco;* 1 Non-Comet: 1 *coco*

Now if the comet platyfish, *CoCo*, is mated to a swordtail which does not have the comet pattern, *coco*, their $F_1$ hybrids develop an entirely new pattern, known as the wagtail pattern which is strikingly different from the comet (Fig. 2).



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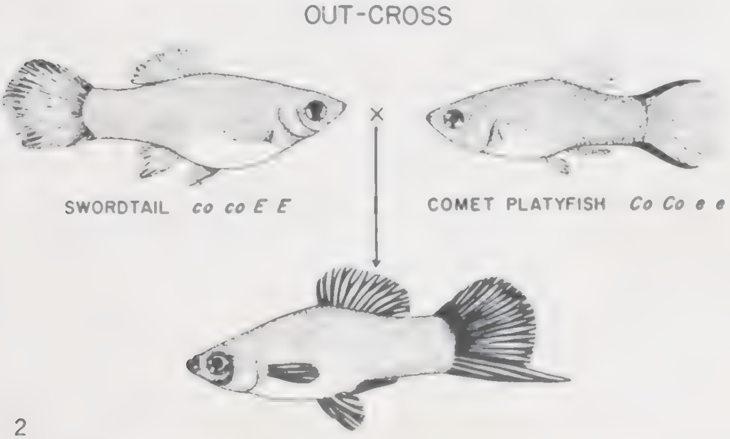

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Fig. 1. A comet platyfish mated with a noncomet member of its own population, or strain, produces young having the dominant comet pattern. The nature of the comet pattern in the offspring is essentially the same as in the parent.

Fig. 2. A comet platyfish mated with a noncomet member of a different species, or strain (in this case, a swordtail), produces young having a radically different color pattern, one that is known as the "wagtail." This change is brought about by the interaction of the dominant comet gene *Co* with a dominant modifier *E*. *E* is a gene which "extends" the action of the *Co* gene. Together *Co* and *E* produce the wagtail.

When two $F_1$ wagtail platyfish–swordtail hybrids are mated, brother to sister, the following types and their ratios appear in the second generation: 9 wagtails, 3 comets, 4 no pattern.

These results were explained by Gordon on the basis of the interaction of two dominant genes. One, the comet gene (*Co*) of the platyfish is readily expressed. The other, an *extensor* of the effect of *Co*, the modifying gene (*E*) of the swordtail, has no visible effect by itself, even though it is a dominant. Gene *E* can only be detected in the presence of the *Co* gene.

In the development of the wagtail pattern, the blackening of parts of the body never reaches a pathological stage. This is because only small black pigment cells (micromelanophores) are involved. Small melanophores in great numbers appear, also, over the entire bodies of the melanistic phases of the mollie, *Mollienesia*, and the mosquito fish, *Gambusia*, but the genetics of these two species have not been studied sufficiently. In the mollies, however, Gordon (1943) showed that when the totally black form is mated to an albino, the first generation fish are intermediate in coloring, being heavily speckled with dark clusters of small melanophores.

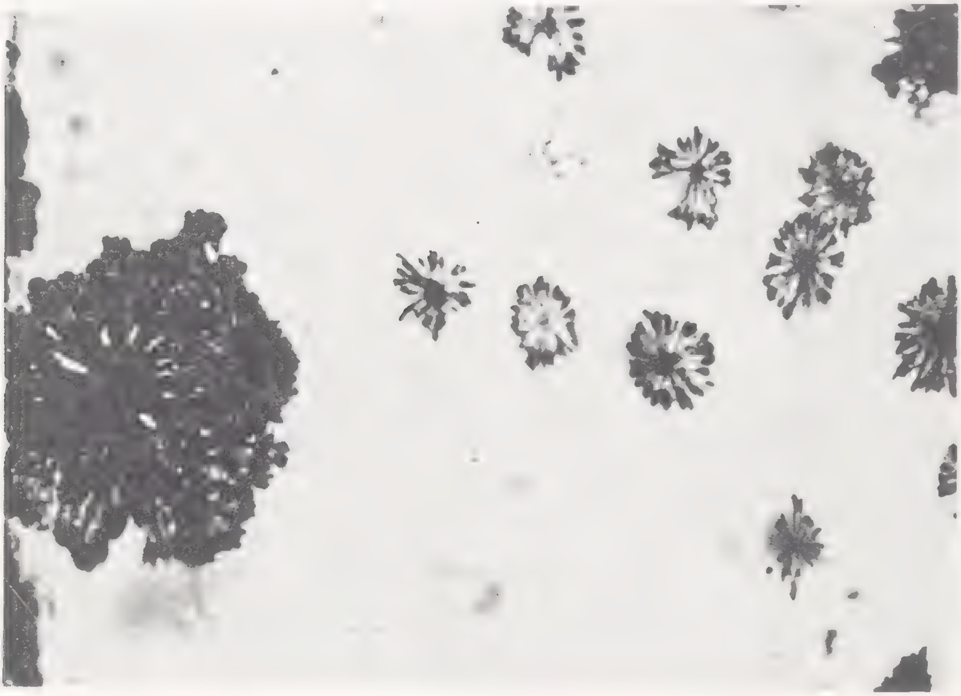

FIG. 3. A normal macromelanophore (to extreme left) and a number of normal micromelanophores (right) in the skin of a spotted platyfish. The large cells are inherited and referred to a sex-linked gene; the smaller cells are inherited by means of an autosomal gene. In platyfish-swordtail hybrids the macromelanophore gene is necessary for the initiation and development of melanomas, the micromelanophore gene is not.

### E. Interaction between Two Pigment Cell Genes

In an earlier paper, Gordon (1928) described the interaction of two pigment cell genes (*Sp* for spotting and *St* for stippling) which produced a series of four distinct effects upon the frequencies of two types of melanophores, one small, the other three times as large (Fig. 3). He expressed this in Table III.

TABLE III
INTERACTION BETWEEN TWO PIGMENT CELL GENES

| Platyfish | | Frequencies of Melanophores in Per Cent | |
|---|---|---|---|
| Phenotype | Genes | Micromelanophores | Macromelanophores |
| Spotted, gray | *Sp* [*] *St* [†] | 70 | 100 |
| Spotted, golden | *Sp* + [‡] | 0 | 30 |
| Gray | + *St* | 100 | 0 |
| Golden | + + | 0 | 0 |

[*] *Sp* is a dominant sex-linked gene for the production of macromelanophores.

[†] *St* is a dominant autosomal gene for the production of micromelanophores.

[‡] + represents the recessive gene to *Sp* and *St*.

An even greater inhibiting effect of the gray gene in its recessive form upon the expression of another dominant gene for macromelanophores was described by Gordon (1938) in the so-called montezuma platyfish–swordtail hybrid which at that time was referred to the gene *Mo*. Later (1948) Gordon showed that the large pigment cells were properly referred to the *striped* gene (*Sr*) of the platyfish.

*Sr St* hybrids are heavily pigmented with macromelanophores while *Sr* + fish, except for a few clusters of large melanophores near the head, are free of macromelanophores. By mating an *Sr* + fish to an + *St* fish, the *Sr St* offspring regain their full complement of macromelanophores.

## IV. GENES INFLUENCING ATYPICAL PIGMENT CELL GROWTH

### A. Frequency and Comparative Pathology

Gordon (1948) in a summary of the frequency of melanomas in fishes living under natural conditions revealed that they were found in representatives of ten orders of elasmobranchs and teleosts. Comparative pathologists who have studied the black pigment cell tumors of fishes, amphibians, reptiles, birds, and mammals have discovered that the *melanocyte* is the common cell type of melanomas of vertebrate animals, including those of man. Grand, Gordon, and Cameron (1941) indicated that the living melanocytes from the melanomas of man, mouse, and fish apparently are similar morphologically. In tissue cultures, the fish melanoma resembles human melanoma more than it does the Harding-Passey mouse melanoma in that its excessive growth is accompanied by an increase in alkalinity to the extent of inhibiting the growth. A com-

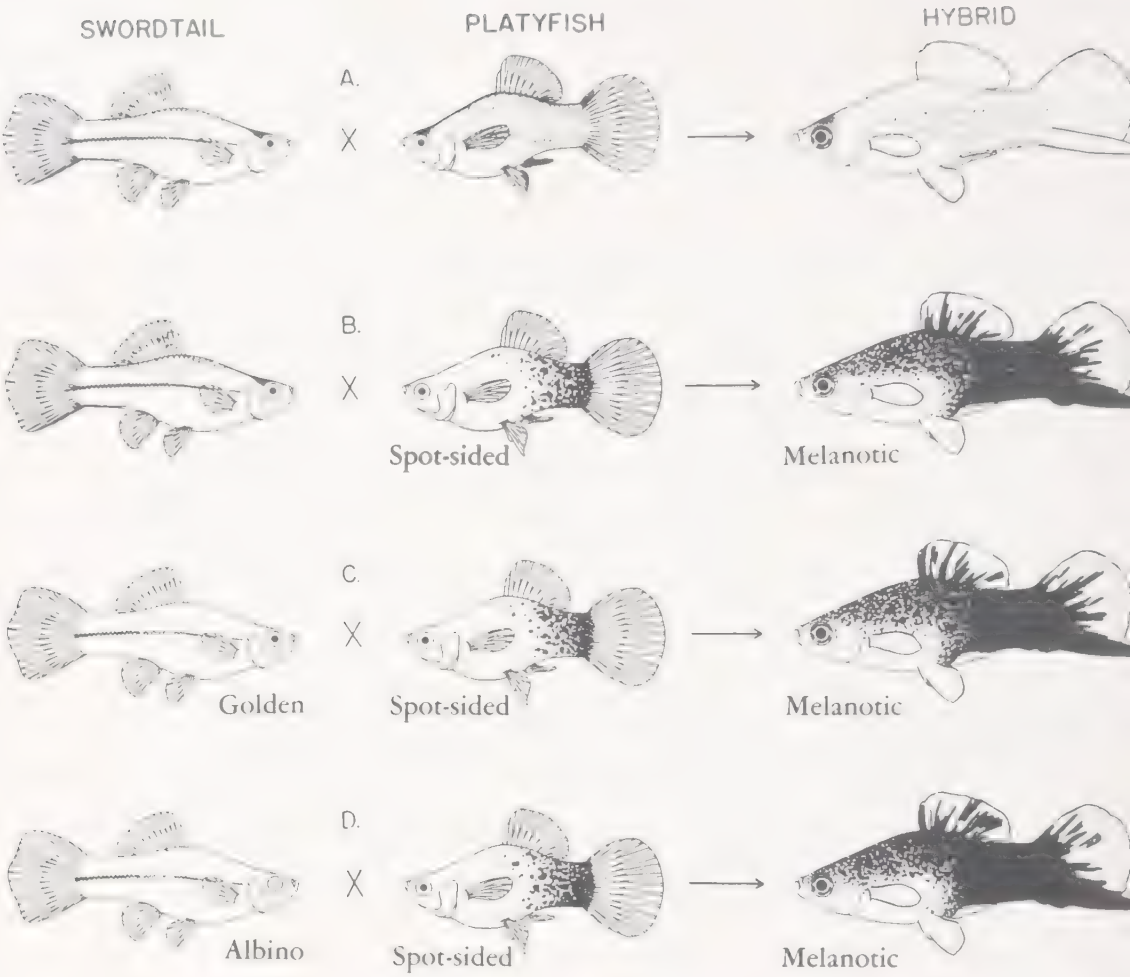

FIG. 4. Micromelanophores versus macromelanophores in the initiation and production of atypical pigment cell growth leading to melanomas in platyfish–swordtail hybrids. Mating A: A gray swordtail with micromelanophores is mated to a gray platyfish with micromelanophores. Result, the hybrid has micromelanophores only, the pigment cell growth in these hybrids is normal. Mating B: A gray swordtail with micromelanophores is mated to a spotted platyfish with macromelanophores only. Result, the hybrid has micromelanophores and macromelanophores. The abnormal growth of the large pigment cells in the hybrid leads to the development of melanomas. Mating C: A golden swordtail without micromelanophores is mated to a spotted platyfish with macromelanophores only. Result, the hybrid has macromelanophores only, yet the hybrid is as susceptible to melanomas as the one from mating B. Mating D: An albino swordtail which has no visible black pigment cells anywhere is mated to a spotted platyfish with macromelanophores only. Result, the hybrid has macromelanophores only. The macromelanophores in their atypical growth produce a melanoma eventually. From these experiments it has been concluded that the macromelanophores rather than the micromelanophores must be present in a platyfish–swordtail hybrid to produce atypical pigment cell growth. Since macromelanophores are inherited and referred to a sex-linked, dominant gene, the melanomas they initiate may be attributed to hereditary factors. The primary gene for the macromelanophores is contributed by the platyfish and the macromelanophore growth modifying genes are contributed by the swordtail.

parative study of the enzyme reactions of melanomatous tissues from mammals and fishes revealed that both are quite similar with respect to tyrosinase activity, according to Fitzpatrick *et al.* (1950).

The analysis of the progressive growth of melanomas in platyfish swordtail hybrids (*Xiphophorus*) by Gordon and Smith (1938) indicated that increasing numbers of macromelanophores first accumulated in the dermal tissues. These eventually replaced the normal tissues, producing a state of melanosis. Then large black pigment cells invaded the deeper body areas by following the paths along the muscle septa. They surrounded the muscle fibers and destroyed them. Nodular lesions, leading to formation of melanomas, usually arose in these primary zones of diffuse melanosis. During the early stages of the disease, macromelanophores were the principal cells observed. As the melanoma progressed, melanocytes increased and macromelanophores decreased because melanocytes no longer differentiated into macromelanophores. Observations by Ermin and Gordon (1955) of regenerating tissues following amputations of melanomas in the dorsal fins made it possible to trace the transformation of melanocytes into macromelanophores. In some measure, the ontogenetic relationships of these pigment-forming cells have been confirmed from observations of transplanted fish melanomas by Marcus and Gordon (1954) and by Greenberg *et al.* (1956).

### B. Genic Imbalance for Melanomas in Hybrids

The influence of genic imbalance has been clearly demonstrated in the development of melanotic tumors in fishes by a series of breeding experiments involving the platyfish and swordtail.

In the natural populations of the platyfish (*X. maculatus*) black-spotted color patterns are found in about 20% of the members. The spotted patterns may be referred to a series of dominant sex-linked alleles while the unspotted condition is the recessive. Gordon (1947a) has shown that sex linkage is incomplete. The spotted pattern is produced by many, unusually large, black pigment cells containing melanin granules, the *macromelanophores*. They are distinguished from the smaller black pigment cells or *micromelanophores* which are inherited autosomally. The term *melanophore* denotes a specialized pigment-bearing cell which is usually found in lower vertebrates. Melanophores and their precursors, the *melanocytes*, are capable of synthesizing melanin granules and have the property of dispersing their pigment from the center of the cell to its periphery and reversing the process. The melanophore is *not* a *macrophage*. These terms and definitions were recommended at the Third Conference on the Biology of Normal and Atypical Pigment Cell Growth (Gordon, 1951a–c).

When a spotted (macromelanophore-carrying) platyfish was mated with a related, but distinct species, the swordtail (*X. helleri*), the $F_1$ spotted platyfish–swordtail hybrids developed definitive melanomas. On the other hand when an *unspotted* platyfish was mated with a swordtail, the $F_1$ platyfish-swordtail hybrids received no macromelanophores and they never developed melanomas (Fig. 4).

By mating a melanomatous $F_1$ platyfish–swordtail hybrid back to a swordtail it was found that half of their offspring were heavily black-

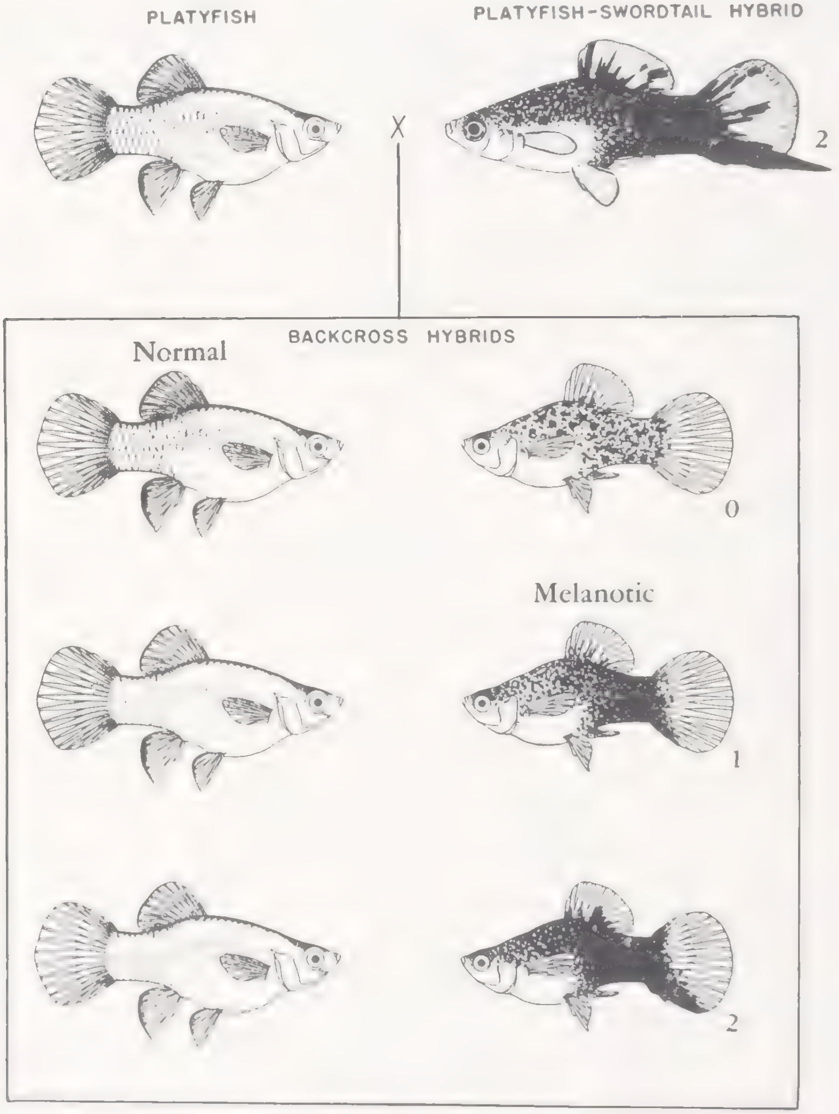

FIG. 5. The intensification and the regression of atypical pigment cell growth is genetically controlled. The mating of a melanotic platyfish–swordtail hybrid ($F_1$) back to the platyfish. Result, the atypical growth of macromelanophores in the spotted hybrids is in general *less* intense than in the spotted $F_1$ parent. The numerals refer to the intensity of atypical pigment cell growth 0, 1, or 2.

spotted with macromelanophores at birth, these fish developed melanomas. The other half were unspotted; they did not develop the disease.

The experiments clearly indicate the positive influence of heredity in the development of melanomas in platyfish–swordtail hybrids. Melanomas are always associated with the presence of macromelanophores, cells which are susceptible to neoplastic activity when in an appropriate genetic environment. Stated in genetic terms, when the macromelanophore gene of the platyfish is in association with macromelanophore-growth-modifying genes of the swordtail a genic imbalance is established in a platyfish–swordtail hybrid which initiates the atypical growth of macromelanophores.

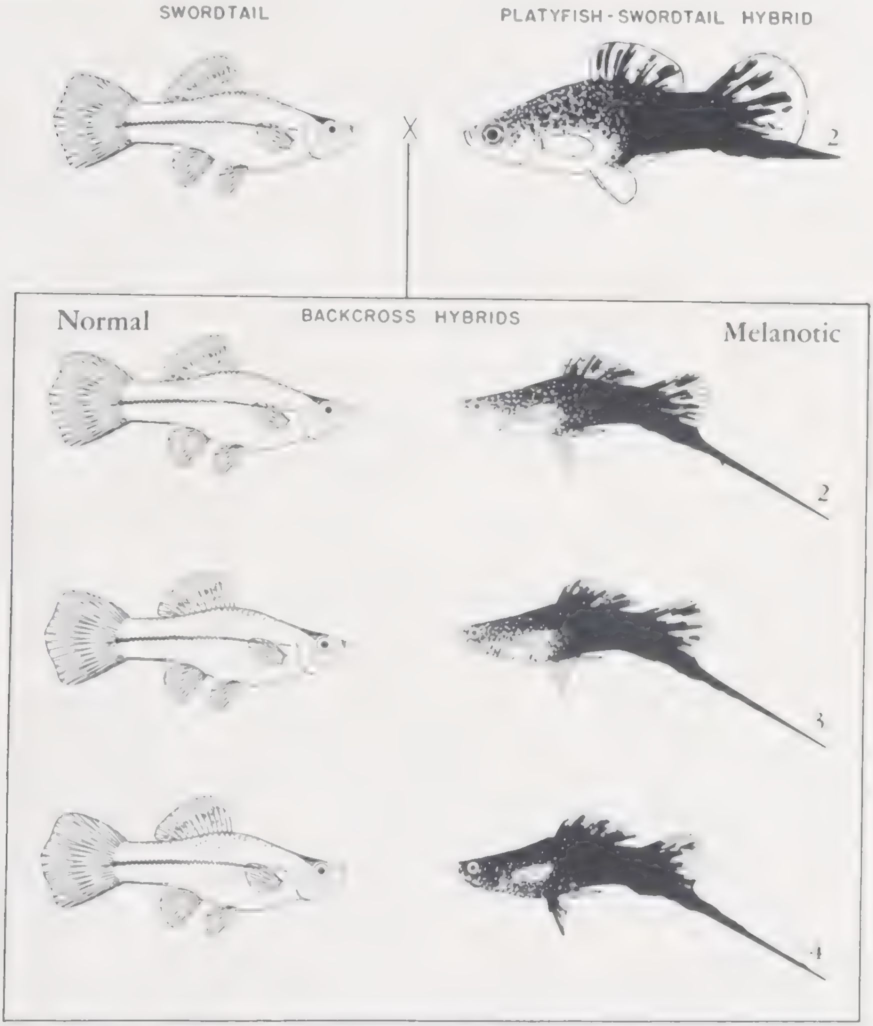

FIG. 6. The mating of a melanotic platyfish–swordtail hybrid ($F_1$) back to the swordtail. Result, the atypical growth of macromelanophores in the spotted hybrids is generally *more* intense than in the spotted $F_1$ parent. The numerals refer to the intensity of atypical pigment cell growth, 2, 3, or 4. For explanation, see text.

Consider the environmental influences surrounding the embryonic development and early life particularly of the backcross hybrid fish obtained from the matings of a heavily spotted hybrid male and a normal swordtail female. The swordtail's ova were fertilized by sperm produced by the melanotic $F_1$ hybrid male. As a result all the embryos developed within the ovary of a normal, healthy swordtail so that all had an identical ovarian environment for about 30 days, during the period of their gestation. At time of parturition, all of the hybrids, spotted and nonspotted, were released within a few hours into the water of a common aquarium and were fed an identical diet. Thus, their pre- and post-natal environments were as identical as could have been made; yet about half of them were diseased and about half were completely normal.

### C. Multiple Factors in the Abnormal Growth of Macromelanophores

As an aid in visualizing the genetic process involved in the production of melanotic neoplasms by hybrids between the platyfish and swordtail, Kosswig (1931) and Gordon (1937, 1951a–c) employed the concept of the multiple factor hypothesis. The essential hereditary factor contributed to the melanotic hybrid by its platyfish parent is the sex-linked, dominant factor *Sp*, for macromelanophores. The swordtail parent transmits to its abnormally pigmented hybrid offspring at least two dominant genes which interact with and modify the normal expression of the *Sp* factor of the platyfish. Since these genes have no visible expression in the swordtail parent, they are referred to simply as *A* and *B*. The following genetic formulas may represent the normal parents involved and their melanotic $F_1$ hybrids:

$P_1$ Swordtail, *spsp AA BB* × Platyfish (Spotted), *SpSp aa bb*
$F_1$ Melanotic Platyfish–Swordtail Hybrids, *Spsp Aa Bb*

Using the multiple factor concept as a basis, certain calculations may be made to reflect the results obtained in two series of backcross experiments: one in which the $F_1$ hybrid is backcrossed to the platyfish, the other to the swordtail (see Figs. 5, 6).

### D. Interracial Matings and Their Effect upon the Expressivity of Pigment Cell Genes

About 10,000 platyfish with and without macromelanophores have been collected in their natural habitats and studied carefully. These native-born fish show no atypical pigment cell growth. Apparently the genetic constitution of the feral platyfish is such that the macromelanophore genes in the spotted platyfish are "buffered" by pigment cell growth modifying genes which prevent atypical growth of these cells, a situation that has probably been brought about by natural selection (Gordon, 1950b).

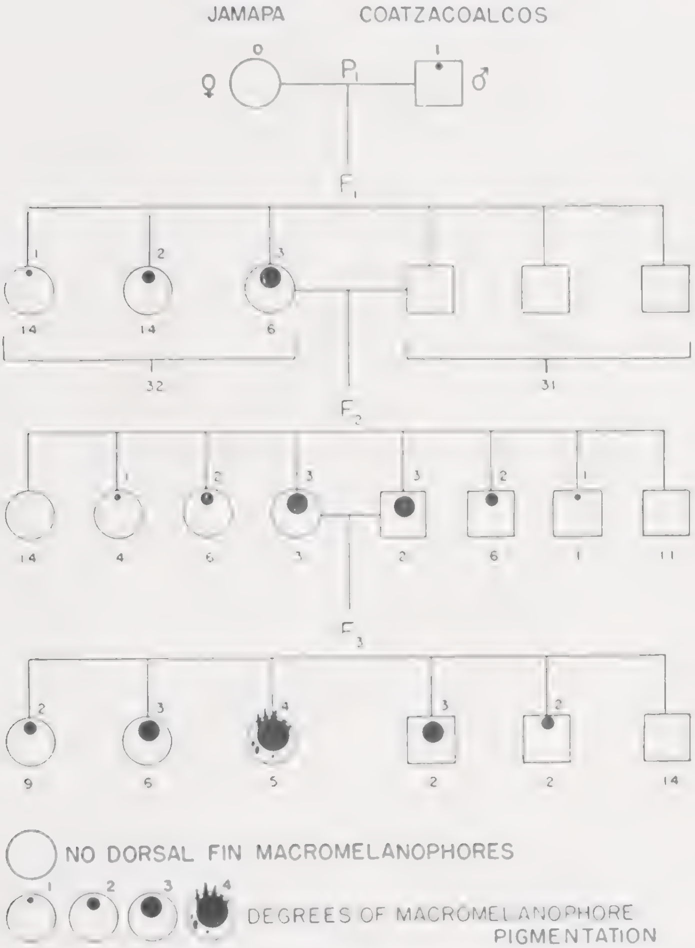

FIG. 7. The effects of outcrossing followed by inbreeding on the atypical growth of macromelanophores involving the *spotted-dorsal* gene, *Sd*, in matings between members of two geographical populations of the same species of the platyfish, *Xiphophorus maculatus*. In the third generation (F$_3$) some platyfish appeared with abnormal growths of macromelanophores so intense that they caused a breakdown of the dorsal fin tissues. This type of abnormal growth parallels the precancerous stage in other hybrid fishes.

Brought to the laboratory, the spotted (macromelanophore carrying) wild fishes from any one given wild population produce offspring that are spotted, or not, in accordance with the principles of incomplete sex-linked inheritance. Their spotted offspring retain the "balanced" genetic constitution of their parents. This continues as long as a spotted parent of one given wild population is mated to a member of its *own* population. On the other hand, if a spotted member of one wild population is mated to a member of a different wild population, the growth of macromelanophores of their hybrid offspring in some instances is suppressed, in other instances it is enhanced to a pathological degree.

The genetic analysis of the phenomena presented by Gordon (1951b, 1954a) is concerned with a study of the role of pigment cell growth modifiers, some of which suppress while others enhance the growth of macromelanophores. An example of the results obtained by matings between two representatives of the same species but from two different natural populations, one from the Río Jamapa, the other from the Río Coatzacoalcos, follows:

$P_1$ Río Jamapa × Río Coatzacoalcos
Female, $(X)Sr(X)Sr$ Male, $(X)Sd(Y)+$

In Fig. 7 which represents the results of this mating only the history of the *Sd* gene for *spotted dorsal* will be traced because the *Sr* gene is hardly modified in its expression.

Note that the *Sd* gene in the $F_1$ hybrids is found only in the 32 daughters; the 31 males are unspotted—a typical criss-cross behavior for a sex linked gene. Note also in the females that the *Sd* gene is variously expressed 1, 2, 3.

When one of the most intensely pigmented $F_1$ daughters (3) was mated to its unspotted brother, they produced in the $F_2$ 13 variously spotted females and 9 variously spotted males; 14 females and 11 males were unspotted. This produces a total of 22 spotted to 25 unspotted $F_2$, a typical backcross ratio of 1:1.

When the most intense-spotted pair of $F_2$ (*Sd*) hybrids were bred, brother to sister, they produced in the $F_3$ 20 females, all of which were spotted, 5 intensely so. Only 4 of the 18 $F_3$ sons were spotted. These results confirm the sex-linked nature of the *Sd* gene.

The 5 females which had intense pigmentation, indicated by the degree 4, were in a state of melanosis. Histological sections of the melanotic fins revealed the destruction of the underlying musculature. No definitive melanomas were, as yet, developed in this or other intraspecific hybrids comparable to those that develop in spotted interspecific hybrids. In the latter, melanosis precedes the development of melanomas. Consequently the atypical growth of macromelanophores that produce the precancerous state in intraspecific hybrids is the same as in the interspecific hybrids.

In another mating between members of the same populations the expressivity of the *Sd* gene was reduced to zero, but it nevertheless was capable under suitable genetic conditions eventually to express itself in terms of a melanotic tumor. In the experiments that follow, the first mating was concerned with a male that was homozygous for the *Sd* gene.

| | | | |
|---|---|---|---|
| P$_1$ | Río Jamapa Female<br>Stripe-sided<br>$(X)Sr(X)Sr$ | × | Río Coatzacoalcos Male<br>Spotted-dorsal<br>$(X)Sd(Y)Sd$ |

All the 150 F$_1$ fish had the stripe-sided trait (*Sr*). Most of them also had the spotted-dorsal character (*Sd*), that is, all but 13 out of a total of 150. Since 137 out of the 150 individuals exhibited the *Sd* pattern, the penetrance of the *Sd* gene may be said to be 91.3% in this mating. The expressivity of the *Sd* character was extremely variable, ranging from zero, as represented by the 13 individuals just mentioned, to a stage far beyond normality, a stage in which the entire fin is blackened and the macromelanophores "spill-over" from the dorsal fin to the more ventral areas of the body. Seven classes of pigmentation intensity were set up arbitrarily to estimate the variability of the *Sd* trait (in brood No. 291):

| | | Frequencies of F$_1$ | |
|---|---|---|---|
| Intensity | Spread of Macromelanophores | Females $(X)Sr(X)Sd$ | Males $(X)Sr(Y)Sd$ |
| (1) | None | 0 | 13 |
| (2) | One small spot on dorsal fin | 0 | 21 |
| (3) | One-half of fin covered | 0 | 20 |
| (4) | More than one-half covered | 13 | 10 |
| (5) | "Spilling-over" slight | 36 | 11 |
| (6) | "Spilling-over" intermediate | 22 | 0 |
| (7) | "Spilling-over" intense | 4 | 0 |
| | Total | 75 | 75 |

These results may be discussed with reference to penetrance and expressivity. Gordon (1947a) has shown that in 9 experiments in which the action of the *Sd* gene from the Rio Jamapa platyfish could be traced, the *Sd* gene has 100% penetrance. Gordon (1951b) showed that while the penetrance for the Río Jamapa platyfish gene *Sd* falls to 91.3% in the Rio Jamapa–Rio Coatzacoalcos hybrid platyfish, it falls to only 1% in the Río Jamapa–Belize River hybrid platyfish.

The penetrance of the *Sd* gene seems to be influenced by sex as well as by its specific gene modifiers. For example, while the value of penetrance of the *Sd* gene is 100% in the females of the Rio Jamapa–Rio Coatzacoalcos hybrid platyfish, it is only about 83% in the males. At the same time, as the value of this gene's penetrance falls in the brood as a whole, the range of its expressivity increases, that is, it varies from no visible effect to an exceptionally strong visible effect.

A male was selected, one out of the thirteen that had no visible macromelanophore on the dorsal fin but was presumably *Sd*, and was mated to a swordtail from the Belize River stock. It was significant that half of the $F_1$ platyfish–swordtail hybrids had strong to intense macromelanophore pigmentation on their dorsal fins and on adjacent parts of their bodies. This clearly showed that the *Sd* gene of the platyfish parent had been completely suppressed but was intact and unaltered.

These results and others indicate that members of differing geographical populations of the platyfish have various macromelanophore-modifying genes, some of which enhance, others suppress the growth of the large black pigment cells. In some geographical platyfish hybrids, the number of macromelanophores may be reduced to zero. In others the pigment cells are stimulated and the early stages of melanosis are indicated. If geographical platyfish hybrids of the first generation are inbred, some of the second generation hybrids (Fig. 7) develop definite manifestations of melanosis. The histological sections reveal that the macromelanophores have invaded the subcutaneous regions of the body and have surrounded and replaced muscles. From these results the conclusion may be drawn that fundamentally there is no difference in the reaction between the *interracial* hybrid members of two geographical populations, and the *interspecific* hybrids with reference to their atypical macromelanophore growth.

### E. Erythromelanomas and Spacial Relationship of Color Genes

Tumors in fishes involving the red pigment cells, xanthoerythrophores, are rare. Rarer still is the phenomenon of the invasion and replacement of one kind of tumor by another, pigmented or not. Gordon (1950a) described the genetics of a mixed pigment cell tumor involving red and black pigment cells, an erythromelanoma in platyfish–swordtail hybrids (Fig. 8). Later Nigrelli, Jakowska, and Gordon (1951) demonstrated histologically and cytologically that two kinds of pigment cell tumors were involved; first, a deep red tumor, an erythrophoroma of the head region and, second, a black tumor, a melanoma of the tail region. When joined, the two pigment cell tumors produced an erythromelanoma.

The genetic history of the erythromelanomas which developed in a significant proportion of a single brood of platyfish–swordtail hybrids was traced to a mating between an exceptionally colored male platyfish and an albino swordtail female. The male playfish had an unusual combination of sex-linked color genes: *Rt* for ruby-throated (erythrophores) and *Sp* for spot-sided pattern (macromelanophores). The genes, *Rt* and *Sp*, were linked on the same chromosome and in this arrangement they were carried in the same gamete. When the genes *Rt* and *Sp* were not linked on the same sex chromosome no erythromelanomas were produced in the hybrids. These details indicate the preciseness of the genetic (chromosomal) control of pigment cell development. They also show the importance of studying the various genes and gene combinations and their specific developmental effects upon the normal and atypical growth of specific cells. From these experiments it may be concluded that the spacial relationship of some genes as well as specific gene combinations influence the development of specific tumors.

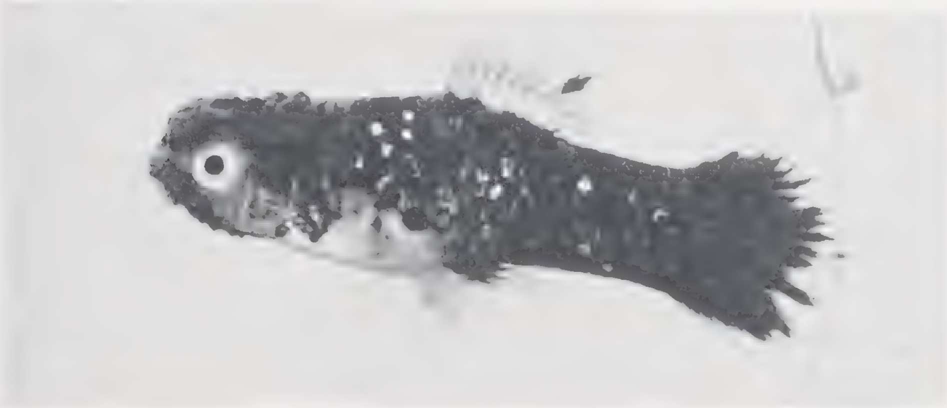

FIG. 8. An erythromelanoma in a platyfish–swordtail hybrid. The development of this rare combination of pigment cell tumor depends upon the interaction of two linked, dominant sex-linked genes of the platyfish and modifying genes of the swordtail.

The manner in which these pigment tumor cells develop has been outlined by Nigrelli, Jakowska, and Gordon (1951). In the early stages of development of the *RtSp st* platyfish–swordtail hybrids, the *Rt* gene and its modifiers influence the growth of red pigment cells in the anterior part of the body, while the *Sp* gene and its modifiers determine the growth of macromelanophores in the posterior regions. As the *RtSp st* hybrids develop, the red pigment cells produce a xanthoerythrophoroma in the opercular area, involving the entire head in some individuals. At the same time the macromelanophores produce a state of melanosis in the caudal peduncle region. By a process of extension of atypical cell growth—mainly in the corium and, to a lesser degree, in the subcutaneous tissues—the macromelanophores and the melanocytes produce a *melanoma* and invade the anterior parts of the body. These two centers of atypical cell growth, the red and the black, eventually produce a number of results. In some hybrids the black tumor cells reach, penetrate, destroy, and replace the red tumor cells and so produce an *erythromelanoma.* In some other hybrids the erythrophoroma of the head remains uninvaded because the macromelanophores do not reach the anterior region. In still other hybrids the macromelanophores apparently grow too rapidly and overrun the head region before the red pigment cells produce a red tumor in that area. This means that a state of general melanosis of the entire body may prevent the development of the erythrophoroma. Thus it may be seen that the genetic control of these tumors in hybrids, all of which carry the *Rt Sp* genes, is not absolutely rigid.

### F. Albino Gene for Amelanotic Melanomas

The cytochemical function of the double recessive albino gene which changes the melanotic melanoma into an amelanotic one (Gordon, 1948) was studied by Greenberg *et al.* (1956). They found that thiol groups are present in the amelanotic but not in melanotic melanocytes. They suggests that the synthesis of melanin pigment granules follows a similar pattern in the amelanotic melanocytes as in the melanotic melanocytes until the 5,6-dihydroxyindole compound is formed. In albino fish there may be an inhibition of oxidation of quinone, thereby preventing the formation of a completely pigmented product. Since the amelanotic melanomas in fish are apparently more neoplastic than the typically black melanomas, the albino gene may have a special function which is to stimulate the growth of the neoplasm. On the other hand it has been shown that in nontumorous fish the albino gene acts as a partial lethal during late stages in the development of the embryos (Gordon, 1942).

## V. EYE CHARACTERISTICS

### A. Cataracts Traced in Five Generations of Platyfish

A blinding opacity of the lens in one eye developed early in a female platyfish while the other eye remained normal and functional. When it was mated to a closely related but normal-eyed male, Gordon (1954b) found that all of their offspring (first generation) had normal eyes. When these normal $F_1$ were mated, they produced 43 $F_2$ fish one of which, a male, had a cataract. The cataractal male was almost completely blind and was unable to mate.

Ten pairs of normal second generation fish were then mated together and subsequently the distribution of the cataractal fish was traced through 5 generations. It was so erratic that no clear cut genetic analysis was possible (Tables IV and V).

### B. The Genetic Basis for the Development of Ocular Tumors

Levine and Gordon (1946) diagnosed a number of exophthalmic ocular tumors that developed in some platyfish, swordtails, and their hybrids as melanomas. They claimed that the melanomas arose from the

TABLE IV

THE REAPPEARANCE OF CATARACTS IN THE THIRD GENERATION OF OFFSPRING FROM TEN MATINGS OF NORMAL SECOND GENERATION PLATYFISH

| Pair Number | I | II | III | IV | V | VI | VII | VIII | IX | X | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Normal eyes | 14 | 21 | 27 | 59 | 34 | 69 | 15 | 20 | 18 | 67 | 344 |
| Cataracts | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 4 |

TABLE V

FIVE BROODS FROM A BROTHER–SISTER MATING OF A CATARACT FEMALE WITH A NORMAL MALE[a]

| Brood Number | I | II | III | IV | V | Total |
|---|---|---|---|---|---|---|
| Normal eyes | 15 | 27 | 18 | 25 | 10 | 95 |
| Cataracts | 11 | 9 | 0 | 0 | 0 | 20 |

[a] These parents are the offspring of Pair Number VIII in Table IV.

pigmented chorioid cells which invaded the retinal and interocular region. Subsequently it was discovered that Jahnel (1939) in Germany had found essentially the same disease in some members of a common brood of platyfish–swordtail hybrids. From the available data Gordon (1946b) suggested tentatively that a recessive gene, *oc* for ocular tumors, interacting with the albino (*i*) gene was primarily responsible for the disease (see Fig. 9).

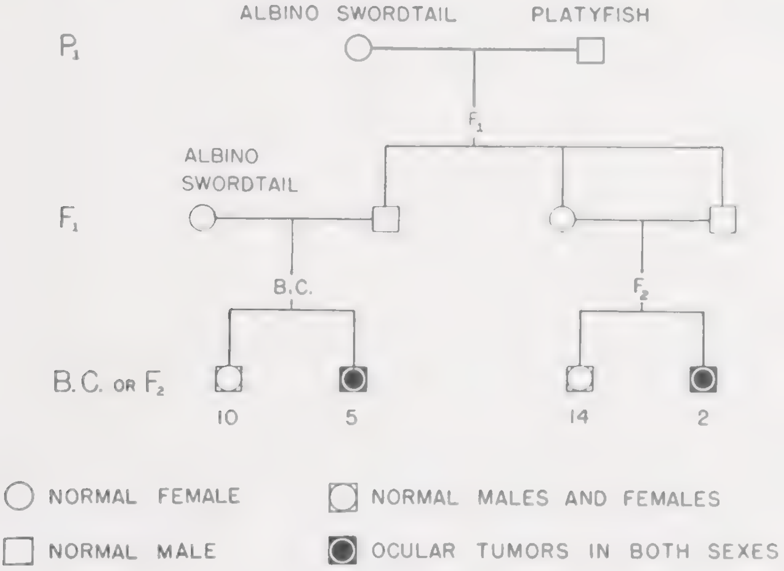

FIG. 9. The inheritance of ocular tumors in swordtail–platyfish hybrids.

### C. Flicker Responses

Differences in the visual sensory apparatus of the platyfish (*X. maculatus*) and the swordtail (*X. helleri*) have been demonstrated by Crozier, Wolf, and Zerrhan-Wolf (1937, 1938). They found that the curves representing the physiological relationship between flicker frequency ($F$) and mean critical illumination ($I_m$) for response to a moving striped pattern were quite different in both form and proportions for each species. The differences in response to flicker and critical illumination between the two species are attributed to genetic factors, which are reflected in the physiological activity of the retinal elements (rods and cones) and central nervous systems.

They tested the black swordtail, a hybrid that looks like a swordtail but which has the $N$ gene for black coloring of the platyfish. It had a quantitative agreement with *X. helleri* in values of $p$ for rods and cones, and in the cones $F_{max}$. On the other hand, the value of the rod $F_{max}$ was similar to *X. maculatus*, and the general level of effective intensities was rather like that of the *X. maculatus*. From their evidence they conclude that the theory of duplicity of vision is correct, the sensitivity mechanism being identified with rods and acuity mechanism with cones. Later Crozier and Wolf (1939a) found that the albino, a recessive mutant of *X. helleri*, showed precisely the same values of critical flash illumination for response to flicker as does the dominant, normally pigmented swordtails. This indicates that retinal pigmentation (and that of the iris) has no influence upon the tested performances. Crozier and Wolf (1939b) then showed that the montezuma swordtail, *X. montezumae*, exhibits essentially the same flicker response as does *X. helleri*. Various color mutants of the platyfish such as gold, black, and red and black-spotted were similar to the ordinary *X. maculatus* and to *X. variatus*. $F_1$ hybrids between *montezumae* and *variatus* showed two *montezumae* characteristics with respect to their cone values. With respect to the values for rods, one feature was like *variatus*, the other was intermediate between the two species. Crozier and Wolf (1939b) also tested *montezumae*, *maculatus* and their $F_1$ and $F_2$ hybrids. They said that the $F_1$ and $F_2$ were statistically equivalent to each other but different from $P_1$. They pointed to the necessity of estimating a capacity for performances in terms of measured performance under defined conditions and offered an example to show how the performances of the parental and hybrid groups vary under varying conditions. They suggested that other genetic analyses be conducted under varying conditions.

## D. Blindness of Cave Forms

The origin of blindness in the *Astyanax mexicanus* (= *Anoptichthys jordani*) population of the Mexican characins, say Breder and Gresser (1941), is rooted in some complex genetic effect. The locomotive behavior of those fish that are able to form an optical image differs from the blind that cannot. Characins with vision will swim together in a compact school while the blind fish continually wander about in a seemingly aimless manner. When eyed and blind fish are both placed in a lighted aquarium, the seeing individuals attempt to school with the wandering blind ones but they are unable to maintain the pace. As a consequence of this upsetting experience the eyed ones frequently attack and kill the eyeless fish.

Fully blind fish are slightly negative to light and have maintained this characteristic after being bred in the light for more than five generations. This negative response has been related to the pineal body. Eyed fish reared under cave conditions become abnormal; one developed a lymphosarcoma (Nigrelli, 1947), others develop hyperplasia of the thyroids, reduction of the relative numbers of pituitary basophiles, reduction in size of the gonads, and an alteration in body form caused by the accumulation of body fat; these and other details are reviewed by Rasquin and Rosenbloom (1954). Hybrids between light-positive and light-negative fishes were found to be intermediate in morphology and reactivity to light by Breder and Rasquin (1947).

The genetic analysis of this complex problem is being continued although the difficulties of mating and rearing the young are great. The problem of determining the precise genetic constitution of the various members is further complicated by the delay in the manifestation of the adult trait, for many individuals are eyed at birth but various degrees of degeneration unfold at some later time. Perihan Sadoglu (personal communication) has been successful in obtaining two series of $F_1$ and $F_2$ young from the mating (1) of blind members of the Chica cave with the eyed river forms, and (2) of blind members of the Sabinas cave (= *Anoptichthys hubbsi*) and eyed river forms. She has also obtained $F_1$ hybrids between blind Pachon cave forms and eyed river fish. Blind fish from the various caves mated together do not yield eyed forms, so that reversion does not occur, apparently the genes for eyelessness and some associated physiological characteristics of cave forms are essentially the same in the various blind populations.

This introduction to the genetics of cave fishes is presented so that the reader may be made aware of the attempt being made to solve an unusual and significant problem in physiological genetics and evolution. In this connection, an apparent, but actually a different problem is involved in the evolution of the blind cyprinid, *Caecobarbus* (= *Barbus*) described by Heuts (1951) from the caves of the Belgian Congo. Still another problem revolves about the eyed catfish *Pimelodella transitoria* and, its eyeless counterpart *Typhlobagrus kronei* described by Pavan (1945, 1946) from the Iporanga caves and rivers in the State of Sao Paulo, Brazil.

## VI. MERISTIC TRAITS

### A. Frequencies of Vertebrae

The intricate interplay of both physiological and genetic influences is clearly demonstrated in Gabriel's (1944) analysis of the factors affecting the number and forms of vertebrae in *Fundulus heteroclitus*. He obtained killifish eggs and sperm samples by stripping females and males and placed both in finger bowls for the fertilization process. The fertilized eggs obtained from 38 sibships were divided into groups some of which were incubated at 24.5, 18.8, and 13.5° C. The larval fish were maintained for a period of 1 to 3 days after hatching, then fixed, studied and the modifications and the frequencies of their vertebrae were compared with those of their parents. This in brief is the method but obviously other important details of the experiments have been omitted in this review.

Gabriel concluded with Hubbs (1922) that temperature may modify the vertebral number through influencing the developmental rate. *Fundulus* eggs developed at the high temperature (24.5° C.) produced fish with low vertebral counts while those kept at 18.8 and 13.5° C. had significantly increased numbers. At the same time, however, other factors being equal, vertebral numbers are subject to genetic control. For example, high parental vertebral numbers are associated with high numbers in the offspring and low parental numbers are associated with low counts in the offspring. Some sibships are temperature stable and are capable of attaining high vertebral counts even at high temperatures.

Gabriel's attempt to determine the temperature-effective period of vertebral frequency sensitivity succeeded only in showing that the number of vertebrae is not yet determined at the 4 to 5 somite stage. Gordon and Benzer (1945) suggested that the sensitive period in vertebral development may coincide with the shift of the higher physiological activity from the anterior part of the embryo to the posterior end which persists for an appreciable interval of time. This suggestion stems from Hyman's (1921) discovery that a secondary and posterior metabolic gradient arises in the developing *Fundulus* embryo during somite formation. In view of Gabriel's work it could possibly be sometime after the fifth somite stage.

In the sea trout (*Salmo trutta trutta*) Taning (1952) stated that the number of vertebrae is determined at the gastrulation stage. At this period the vitelline membrane of the embryo is still impermeable to salts and to water, and its excretory system is not yet functioning. In terms of degree days of development, the sensitive period for the sea trout embryo is between 145 and 165 D°. This is shortly before the hardy "eyed egg" stage; hatching takes place at 400 D°. In his analysis of the relative roles of genetic and environmental influences on meristic characters in the sea trout, Taning suggested that the vertebral frequencies are more under the influence of genetic control because the vertebrae are formed early in the development of the embryo. Traits like the number of rays in the various fins which are determined later in ontogeny are more under exogenous influences that act upon the metabolism of the developing fish. The cold-water trout has a long developmental period, but in the tropical paradise fish (*Macropodus opercularis*) which hatches in 2 days at 80° F. the picture is different. In this warm-water species Lindsey (1954) showed that while the order of fixation of the final count of meristic traits in the different series roughly follows the order of their ontogenetic appearance, the structures are still sensitive to environmental influences (temperature) 20 days after hatching.

## B. Abnormal Vertebrae

Some environmental factors responsible for various abnormalities in the vertebral column of fishes have been described by Gabriel (1944). Genetic factors also play a part in the development of spinal abnormalities in the killifishes. For example, Gordon (1934) found a dwarf female platyfish which had a strongly coiled spinal column. It also had a black spot near the tail which was known to be inherited as a simple Mendelian dominant trait, as shown by Gordon and Fraser (1931). The black-spotted, hunchback female was mated to a normal nonspotted male. In their brood of 64 fish, all showed the black one-spot pattern but only a single specimen had a deformed spine. Then the same hunchback female was mated to another hunchbacked platy of a different stock; 70 $F_1$ young were born but none exhibited any spinal abnormality. Then a pair of phenotypically normal $F_1$ offspring derived from deformed parents were mated; in their brood only one specimen was deformed.

As opposed to these nondefinitive results, Aida (1930) showed that two spinal abnormalities in the Japanese medaka (*Oryzias latipes*), which he called "wavy" and "fused," were each inherited as Mendelian recessives. However, in Aida's experiments the pattern of Mendelian inheritance of spinal deformities was not typically recessive, because when he mated two *similarly* abnormal specimens a few members of the next generation were normal. If "wavy" and "fused" were typically recessive Mendelian traits, no normal individuals should have appeared when fish with similar abnormalities were mated.

Simple Mendelian inheritance of spinal deformities among the guppies, *Lebistes reticulatus*, have been described by Kirpichnikov (1935), Goodrich *et al.* (1942), and Rosenthal and Rosenthal (1950). Crooked back-bones in guppies were induced by Gibson (1954) by subjecting the mother and young-born to water maintained at 32° C.; this is an apparent shock effect.

## C. Frequencies of Fin Rays

With regard to another meristic series, Schmidt (1919) showed that *Lebistes* parents that each had 6 dorsal rays produced offspring that had an average of 6.7 fin rays and parents that each had 8 rays produced young that averaged 7.9 fin rays. He demonstrated that temperature influenced the number of fin rays as well as genetic factors. Nevertheless he was able to select some genetic lines of guppies for high fin ray counts, others for low numbers. From these two parental groups he obtained fish with corresponding high and low ray counts even though they were reared at the same temperature.

Svärdson (1945) mated two guppies each with 7 dorsal fin rays and obtained mostly 7-rayed fish (mean = 7.05). From the brood he selected 2 parents, each having only 6 dorsal fin rays. These produced young with a mean number of 6.944 rays. He kept on selecting for lower numbers of rays and in the fifth generation obtained a brood with a 6.465 mean number of dorsal fin rays. During his experiments he obtained some guppies with only 2 dorsal rays, but these and other marked deviants were generally of low vigor and fertility.

Svärdson also tried to select for 8 or more dorsal rays but although an occasional 9-rayed fish appeared, only slight advance was demonstrated in the mean number of rays. To account for the results he obtained he utilized Mather's (1943) theory of polygenic inheritance. Svardson found no one gene influencing the number of fin rays but cites the work of Golovinskaya (1940) who found a mutant in the carp which it is claimed has a pleiotropic effect in that it reduces vigor of fish, the number of scales, fin rays, and gill rakers.

On the other hand Thompson and Adams (1936) reported that carp, *Cyprinus carpio*, introduced in 1929 in two lakes in Illinois mutated to produce fish totally without pelvic fins or pelves or with much reduced ones. These mutant carp were not only vigorous but appeared to supplant the normal ones. In 1934 they constituted about 30% of this population.

The influence of temperature and salinity on meristic counts has been shown in two distinct populations of the Belgian sticklebacks (*Gasterosteus aculeatus*) which differ in their inherited morphological and physiological characteristics. Heuts (1947) found that population "A" always inhabits fresh water while population "B" is restricted to the ocean for most of its life cycle. "A" is smaller in size; it has a higher number of vertebrae but lower number of integumentary plates. Heuts (1949) reared the eggs of these two stickleback populations until they hatched, under varying temperatures and salinity. Those that survived to the age of four months were killed and examined for the number of rays in the dorsal, anal and pectoral fins. He found that these were modified in "A" and "B", each in a characteristic way in response to both temperature and salinity.

Sticklebacks of population "A" reared in fresh water showed that their dorsal and anal ray counts varied inversely with the temperature but this response did not occur in saline water. On the other hand sticklebacks of population "B" reared in saline water showed variation in the fin ray counts in response to varying temperatures but not when reared in fresh water. The pectoral fin ray count showed a reversed pattern in each of the two groups.

Heuts makes this important point. The two Belgian races of sticklebacks differ in size, vertebral numbers, and lateral plate numbers, but *not* in their fin ray frequencies. But the similarity in their fin ray frequencies is brought about by entirely different, genotypically determined reaction systems. The differences between the two populations in a given character, seemingly alike phenotypically, show up when subjected to genetic and physiological tests.

The influence of heredity in maintaining the identity of two races of the brown trout, *Salmo trutta*, reared in a similar environment was studied by Alm (1949). The large, silvery form *lacustris* was obtained from large Swedish lakes, while the small, brownish form *ferox* was taken from small brooks in forests and mountains. Alm maintained representatives of these two races under similar conditions for more than two generations and found that the $F_1$ and $F_2$ offspring of each race resembled their corresponding parents with respect both to their coloring and to the time of reaching sexual maturity. He concluded that the racial divergences between the large silver and the small brown trout races were chiefly due to genetic factors. Thompson (1930) suggested that isolation is a more effective cause of racial differences within a fish species than differences in environments to which fish may respond directly within one generation or be gradually adapted over many generations. As long as populations are isolated, heritable differences from the original condition, and from each other, will accumulate. If, however, interbreeding is occasionally possible between partially isolated populations, these differences will tend to be ironed out. He found from his study of 17 populations of Johnny darters (*Boleosoma nigrum*) in Illinois that the mean ray number differences between the various populations increases with the distance by water. Because the minimum difference is always near zero, or rather below the value of mathematical significance even in the greatest water distances, he concludes that a randomness of variation exists. These early studies of Thompson fit nicely within some principles of population genetics, particularly Sewall Wright's concept of the influence of genetic drift. This interpretation may also be applied to the 24 isolated populations of the golden shiner (*Notemigonus crysoleucas*) in Michigan lakes studied by Schultz (1926) who found that they represented a recognizable exception to the generalization of Hubbs (1926) that the number of meristic units may be increased in cool waters in which development is retarded and decreased in warmer waters in which development is accelerated. On the basis of 4437 *Oryzias latipes* from 56 localities in Japan, Egami (1954a) found that geographical variations are controlled more by genetic than by environmental factors. The effects of genetic drift probably account for the variability of *Gambusia affinis* in localities in the Escambia River in southern Alabama and western Florida, as measured in part by the frequencies of dorsal and anal rays (Bailey, Winn, and Smith, 1954). Nevertheless Hubbs (1955) has declared that the eastern populations of *Gambusia affinis* differ from those of the western forms in having specifically 10 instead of 9 anal rays and specifically 7 instead of 6 dorsal rays. He asserted that these "taxonomic differences" may be "attributed to the action of a single gene" and "the Atlantic Coast type is completely dominant." These conclusions cannot be accepted because there are no published field or experimental data to support them.

In the $F_1$ hybrids between brook and lake trout (*Salvelinus fontinalis* and *S. namaycush*) Slastenenko (1954) found that certain characteristics like pectoral and ventral fin lengths and heights and size of eye show relative growth relationships which are approximately intermediate, but the head length is never intermediate. In the hybrids between two subspecies of the Johnny darter, *Boleosoma nigrum nigrum* and *B. n. eulepis*, Lagler and Bailey (1947) found that squamation was variously expressed. Squamation of the nape in the $F_1$ hybrids resembled *eulepis* more closely. The squamation of the cheek in the $F_1$ also resembled *eulepis* but less closely. Breast squamation of the $F_1$ hybrids was intermediate but here, too, the values were slightly closer to *eulepis* than *nigrum*. Thus there are exceptions to Hubbs' (1955) assertion that natural interspecific hybrids are precisely intermediate between their parental species in all characters in which those species differ. Additional exceptions of imperfect intermediacy in the $F_1$ hybrids may be found in the platyfish–swordtail hybrids for color patterns described by Gordon (1931, 1946a, 1951c), for embryonic development described by Tavolga (1949), for various structural elements in the gonopodia indicated by Gordon and Rosen (1951), for various items in the sexual behavior patterns demonstrated by Clark *et al.* (1954). As yet we are unready for a generalized statement on the method of inheritance of taxonomic characters.

### D. The Shapes of Some Meristic Characters

Not only are the frequencies of meristic traits in fishes under genetic influence but so are the shapes of some of them. The gonopodia of poeciliid fishes contain a complex of meristic features among which are a number of comb-like segments known as *distal serrae* and a group of spine-like processes. Gordon and Rosen (1951) studied the genetics of these gonopodial segments in the platyfish (*Xiphophorus maculatus*), the swordtail (*X. helleri*), their interspecific $F_1$ and $F_2$ hybrids, and a number of backcross hybrids. They concluded that: (1) the shapes of the gonopodial spines in the $F_1$ hybrids are intermediate between the values for the platyfish and the swordtail; (2) the $F_2$ hybrids have approximately the same mean values as the $F_1$ but their values are more widely dispersed than those of the $F_1$, and some of the $F_2$ hybrids attain the high values of the $P_1$ platyfish, while other $F_2$ hybrids approach closely the low values of the $P_1$ swordtail; (3) the backcross hybrids obtained by mating an $F_1$ hybrid female to a platyfish male have values which approach those of the platyfish and when these hybrids are backcrossed again to the platyfish, the subsequent hybrid fish have values which are even closer to those of the $P_1$ platyfish; (4) the backcross hybrids obtained by mating between an $F_1$ and a swordtail have values in the direction of the $P_1$ swordtail. They interpreted these results on the basis of multiple factors in which the number of factors is small.

## VII. TRANSPLANTATION, RESISTANCE, SEROLOGY

### A. Transplantation

The work in transplantation of normal and cancerous tissues in fishes is still fragmentary. At present the results seem to indicate that the genetic factors are important, but they are by no means the exclusive ones. Goodrich and Nichols (1933) transplanted scales with their adhering dermal tissues containing some melanophores obtained from pigmented areas of the skin of the goldfish into unpigmented areas of the skin. They found that the melanophores persisted and invaded the surrounding unpigmented dermal tissues. They found that when autotransplants were made in goldfish there was no tissue antagonism but that there were incompatibilities in homotransplants. Egami (1954b) successfully transplanted fin rays in the Japanese medaka, *Oryzias latipes*.

but whether these were auto-, homo-, or heterotransplants was not stated.[1]

Held (1950) was successful in making autotransplants of anal and dorsal fins in the platyfish, *Xiphophorus maculatus*, but he failed when he attempted to transfer fins from one individual to its siblings. Kallman and Gordon (1955), following up Held's lead, found that homotransplants as well as autotransplants of anal and dorsal fins were successful in 1 strain of platyfish that had been inbred for 19 generations by brother–sister mating. This pointed to a strong positive genetic correspondence between donor and host. Marcus and Gordon (1954) had previously shown that autotransplants of fragments of the *Sc* melanoma were successful. Homotransplants were also successful in hosts having a common *Sc* (*spotted-caudal*) gene (Fig. 10). Transplants into other hosts either failed completely or were maintained for a short period only.

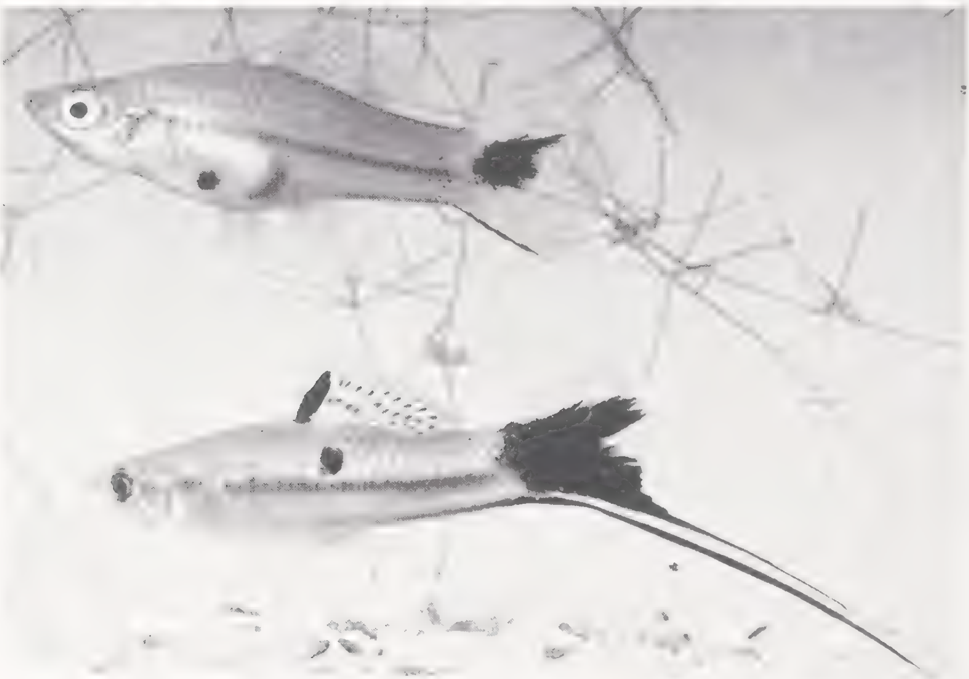

FIG. 10. A successful homotransplant (above) in a female *montezumae-helleri* hybrid swordtail and an autotransplant in a male hybrid swordtail (below). The transplant was an *Sc* melanoma fragment.

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[1] *Autotransplant*, tissue transplanted from one part of the individual to another part of the same individual. *Homotransplant*, tissue transplanted from one individual to another individual of the same species, known also as an *isotransplant* when individuals are of the same highly inbred strain. *Heterotransplant*, tissue transplanted from one species to another species or to a hybrid between the two species.

The first success in transplanting a fragment of a platyfish-swordtail melanoma into the anterior chamber of the frog's eye was recently reported by Giovanella and Pecini (1954). Only one of several attempts succeeded. The tumor was observed for 27 days and during this period the melanoma cells penetrated the iris of the frog.

Humm and Young (1956) have been able to transplant neural tube tissue from platyfish-swordtail hybrids, genetically destined to develop melanomas, into swordtail embryos. In two instances macromelanophores appeared in the swordtails. Transplants taken from hybrids without neural tube tissue did not produce melanophores in host swordtail embryos. Humm and Clark (1955) and Greenberg *et al.* (1956) have studied the enzymology, metabolism, and cytochemistry of the typical and amelanotic melanomas. This work should open up new studies of the cell physiology of genetically induced tumors of fishes.

## B. Temperature Tolerance

Fry and Gibson (1953) maintained male speckled trout (*Salvelinus fontinalis*) and female lake trout (*Salvelinus namaycush*) and their hybrids for certain lengths of time at a constant temperature for acclimatization and then placed them directly into baths held at various test temperatures. The time required to kill the fishes was recorded. All fishes were equally hardy at 0° C. when acclimated to 20° C. At the lowest acclimation at 10° C., the position of the hybrids was approximately midway between those for the two parents. At 15° C. acclimation the hybrids approached the median resistance times of the brook trout parent more closely; at 20° C. acclimation, this trend was even more pronounced.

From a single gravid female guppy (*Lebistes reticulatus*) Gibson (1954) obtained young which by various systems of mating were used to produce three inbred lines (*a*, *ad*, and *b*) each of which was tested, as was a totally unselected stock, for their upper lethal temperatures. She found that inbred lines *a* and *ad* showed a lower resistance than either *b* or unselected guppies. Hybrid progeny of lines *a* and *b* had a much longer resistance to 37° C. than *a*, *b*, or unselected fish. Hart (1952) studying many fish species concluded that in some the lethal temperature patterns are inherited because he found them constant throughout their range.

## C. Resistance to Infections, Rate of Growth, and Maturity

Embody and Hayford (1926) showed that by methods of selective breeding of brook trout, *Salvelinus fontinalis*, they were able to reduce their mortality in response to infection by "*Bacterium trutta*" and "*Octo-*

*mitus salmonis*" from 98% to 30.8% in 3 generations. During this process the average length of July fingerlings increased from 2 to almost 4 inches. Later in a progress report, Hayford and Embody (1930) indicated that after 5 generations of selective breeding of brook trout, the following were the results:

(1) Increase in the rate of growth and size of fingerlings. The larger breeders produce more eggs at the first and second spawnings.

(2) Increase in the mortality during the eyed stage of developing embryos.

(3) Increased resistance to furunculosis and to actomitiasis, but not to *Gyrodactylus, Chylodon,* and some gill bacteria.

The authors pointed out some of the practical advantages that have been attained. For example, trout for stocking purposes have averaged between 6 and 7 inches at the end of the first summer instead of the usual 3 to 5 inch fingerlings. The increased egg production has made it possible to keep only about 25% of the number of breeders to obtain the same number of eggs as formerly.

Davis (1931) reported that through selective breeding certain stocks of trout, *Salvelinus fontinalis*, were induced to spawn regularly within a short period year after year. One lot was ready to spawn at the Pittsford, Vermont hatchery on October 29 the first year, and on October 25 the second year. This and other examples led Davis to conclude that heredity plays an important part, as well as temperature, in the determination of the time of spawning of trout.

Wolf (1954) collected eleven different lots of brook trout, *Salvelinus fontinalis*, and eight lots of brown trout, *Salmo trutta*, fingerlings from various hatcheries in northeastern United States in another effort to obtain disease-resistant fishes by genetic methods. The fish represented brood stocks which had existed as separate strains in various state hatcheries for at least eight years. At the State Fish Hatchery at Rome, New York, the various fish were maintained by Wolf in separate enclosures but they were fed the same diets and kept as nearly as possible under the same conditions. They were exposed equally to infection with both furunculosis (*Bacterium salmonicidia*) and ulcer disease (*Hemophilus piscium*). Wolf isolated bacteriological cultures from the fish at frequent intervals to verify the presence of the diseases. He fin-clipped the moribund fishes for identification and then spread them around in all the tested groups. Those which died from the diseases were ground up and the minced fish were fed to experimental trout.

Wolf found that the various trout strains differed greatly in susceptibility to infection. For example, in 9 out of 11 strains of brook trout tested over a period of 11 months, furunculosis and ulcer disease killed

74 to 100% of the fishes. In 1 strain the losses amounted only to 24% and in the most resistant strain deaths were as low as 5%. From these results Wolf concluded that he had attained a measure of the positive effects of genetic selection, but he indicated that the trout's resistance to disease depends upon a complexity of physiological factors.

Snieszko (1954b), organizer of the first Symposium on Fish Diseases (1954a), reported that at the Leetown, West Virginia, station, brook trout suffered such heavy losses from furunculosis that production of this species of trout became impractical, although the best known treatments were employed. Snieszko decided to extend Wolf's experiments in the selective breeding of trout for disease resistance. He compared the susceptibility of the trout used for years at Leetown with a stock found to be most resistant by Wolf, together with another fairly resistant one from a nearby hatchery in Maryland. His observations as of October 1, 1954 also indicated considerable differences in the susceptibility of the 3 strains of brook trout to infection with ulcer disease and furunculosis. Losses were either 64, 36, or 19%.

King (1954) regarded genetic selection for resistance as a problem within the scope of our knowledge of population phenomena, for it involves changing the gene frequencies in the selected line. Any gene or group of genes present in high frequency attains its characteristic frequency because it fits well with the other genetic material in its population. In other words, the various elements of the gene-pool are coadapted and are capable of maintaining a dynamic equilibrium. A directional change in the frequency of one or more genes by artificial selection requires compensatory changes in the frequencies of others. Its success depends upon the attainment of a new genic balance that is equally adaptive. The role of genes of both the host and its pathogenic organisms was evaluated by Gowen (1948) who pointed out that a single gene difference is sufficient in some cases (as in *Pasteurella* of the guinea pig) to make the difference between susceptibility or resistance to a disease. In most cases (including typhoid of mice), however, more genes are involved and they present a more complex type of inheritance. The graded expressions of disease resistance, it should be remembered, may arise through differences in either the genotypes of the hosts or those of the pathogens that initiate the disease syndrome (Gordon, 1954b).

## D. Effects of Ultraviolet Radiation

Radiation from a mercury vapor lamp produced different reactions in three genetically different forms of the goldfish *Carassius auratus*. The common goldfish (*TT*) is usually darkly pigmented soon after hatching but it loses its melanophores at about three months of age. The transparent shubunkin (*T'T'*) is capable of developing few if any melanophores. Neither of these two parental forms produce typical melanophores when irradiated. The hybrid goldfish known as calico (*TT'*) is irregularly mottled with patches of melanophores. When irradiated, new areas of pigmentation are induced in the calico. Goodrich and Trinkaus (1939) suggested that irradiation stimulates the transformation of colorless pigment cells in the dermis to definitive melanophores. Perhaps some inhibitor for melanin formation is inactivated as a result of radiation.

### E. Serological Reactions

The serological reactions in four races of carp, *Cyprinus carpio*, were found to differ by Taliev (1946). Compared with the Galician mirror and scaled carp, which are quite close, the Amur River carp was the most distant taxonomically and the Lake Taparavani carp less so.

The blood of $F_1$ hybrids produced by a Galician male and Taparavani female has the highest percentage of antigenic properties; these are more characteristic of the mother than of the father. In addition, the $F_1$ have some new features. The $F_2$ hybrids, some of which resemble $P_1$ mirror carp morphologically, retain the serological peculiarities of the hybrids, that is, they resemble the $P_1$ female.

## VIII. SUSCEPTIBILITY TO THYROID TUMORS

### A. Frequencies and Relationship to Low Iodine Concentration of Water

Spontaneous tumors of the thyroid gland have been reported by Schlumberger and Lucké (1948), Gorbman and Gordon (1951), and Nigrelli (1952) in at least eighteen species of freshwater and marine fishes, all of which have been maintained for long or short periods in aquaria or in fish hatcheries.

Studies of the thyroid tumors in trout (*Salvelinus fontinalis*) by Marine and Lenhart (1910, 1911), Marine (1914), and Gaylord and Marsh (1914) demonstrated the relationship between iodine supply in the water and hyperplastic growth of the thyroid cells. These studies provided much of the background for some of our present understanding of the etiology of human goiters. For example, it was shown that the tumors in trout regressed when iodine was added to the water in which trout were maintained.

In contrast with the encapsulated goiters of mammals, the hyperplastic thyroids of fishes are remarkable for their invasive properties. This property led earlier workers to consider the invasive thyroid tumors as "carcinomas." Thyroid tumors having somewhat similar characters were described in the freshwater swordtail, *Xiphophorus montezumae* by Gorbman and Gordon (1951) (Table VI). Nigrelli (1952) diagnosed the thyroid tumor in the marine killifishes, *Fundulus heteroclitus* and *Cyprinodon variegatus*, as interacinous adenomas, and in the marine angel fish, *Angelichthys isabelita*, as an adenocarcinoma. He suggested that iodine concentration of the sea water may be just one of several factors involved in their etiology; others may be oxygen tension, light intensity, salinity, calcium–magnesium ratios, and enforced lower activity. Pickford (1953), however, reported that thyroidal hyperplasia in *Fundulus heteroclitus* reared in saltwater aquaria can be prevented by the addition of iodine in their food.

## B. Influence of Heredity

The influence of heredity in these seemingly purely physiological and pathological problems has been demonstrated in a series of papers by Gorbman and Gordon (1951), Berg, Edgar, and Gordon (1953), Berg and Gorbman (1953), Berg, Gordon, and Gorbman (1954), and Baker *et al.* (1955). They studied the physiology and genetics of atypical thyroid cell growth in seven laboratory-reared species of the genus *Xiphophorus* which included four species of the platyfish and three of the swordtails. All originally had been obtained from rivers flowing down the Atlantic coast into the Gulf of Mexico and Honduras. Not all of the seven xiphophorin fishes, living under uniform laboratory conditions, developed thyroid tumors in response to the low iodine content of the aquarium water. The thyroid tumors developed at different rates and in different ways in the various susceptible species and in various strains of the same species. This is expressed in Table VII and Figs. 11, 12. The most remarkable of these was the abnormal growth in several strains of the common platyfish, *Xiphophorus maculatus*. These had a high incidence of functional thyroid tumors in the kidneys rather than in the expected thyroid region.

### 1. THYROID TUMORS IN THE MONTEZUMA SWORDTAILS

In *X. montezumae* the thyroid tumors involved the gills, gill arches base of ventral aorta, bulbar region of the heart tongue, and muscles of the lower jaw (Fig. 13). They usually formed nodular bulges, clearly visible externally on the lower side of the head at the isthmus as a pinkish swelling. The tumors were invasive, penetrating muscle, gills, and bones. By gradually filling the visceral arches, the tumorous tissues apparently interfered with respiration and thus contributed to the fish's death. In microscopic details the tumors are not uniform, consisting of areas of

## TABLE VI

### CHARACTERISTICS OF NORMAL AND TUMOROUS THYROID TISSUE IN SPECIES OF WILD AND DOMESTICATED BROOK TROUT AND MONTEZUMA SWORDTAILS [a]

| | Trout | | Swordtails | | |
|---|---|---|---|---|---|
| | Wild | Hatchery | Wild | Laboratory Strains | |
| | | | Río Axtla | “43” | “$38^3$” |
| PRETUMOR | | | | | |
| Hypertrophy of follicle cells | Occasional | Extreme | Occasional | Extreme | Extreme |
| Reduction of colloid in follicle | Normal | Extreme | Normal | Extreme | Extreme |
| Hyperplasia | Occasional | Extreme | Occasional | Extreme | Extreme |
| Hyperemia | Absent | Present | Absent | Present | Present |
| Hyperplasia of connective tissue | Absent | Present | Absent | Absent | Absent |
| TUMOR | | | | | |
| Incidence | Very rare | Endemic at one time | Not seen [b] | 100% | 100% |
| Time of development | Unknown | 4 months | | 6 months | 1 year |
| Regression | | After 3 years | | Unknown | Unknown |

[a] Data from Berg, Edgar, and Gordon (1953).

[b] Among approximately 1000 wild *X. montezumae* swordtails examined.

## TABLE VII

Normal and Atypical Thyroid Cell Growth in Xiphophorin Fishes

| Species | Number of Follicles in Nontumorous Animals |  | External Goiter | Hyper-trophy | Trabe-cular | Micro-follicular | Macro-follicular | Cystic | Invasive | Per Cent Tumors |
|---|---|---|---|---|---|---|---|---|---|---|
|  | Wild [a] | Laboratory |  |  |  |  |  |  |  |  |
| *helleri* | 68–122 | 26–122 | —[b] | II | — | — | — | — | — | 0 |
| *maculatus* | 24–140 | 52–93 | II | III | III | III | III | III | II | to 87[c] |
| *variatus* | 65–175 | 33–135 | — | II | II | II | — | — | I | 27 |
| *montezumae* | 33–108 | —[d] | II | III | III | II | — | I | III | to 100[c] |
| *pygmaeus* | 23–56 | —[d] | I | II | II | III | — | I | II | to 100 |
| *xiphidium* | 25–47 | 41–188 | I | — | — | II | III | I | I | 2 |
| *couchianus* | 23–39 | 121–165 | — | — | — | — | — | — | — | 0 |

[a] Except for this column the data refers to laboratory-reared fishes.

[b] —, absent; I, present; II, frequent; III, intense.

[c] Incidence of thyroid tumor varies in different sublines, see Tables VI and VIII.

[d] No animals without tumors available.

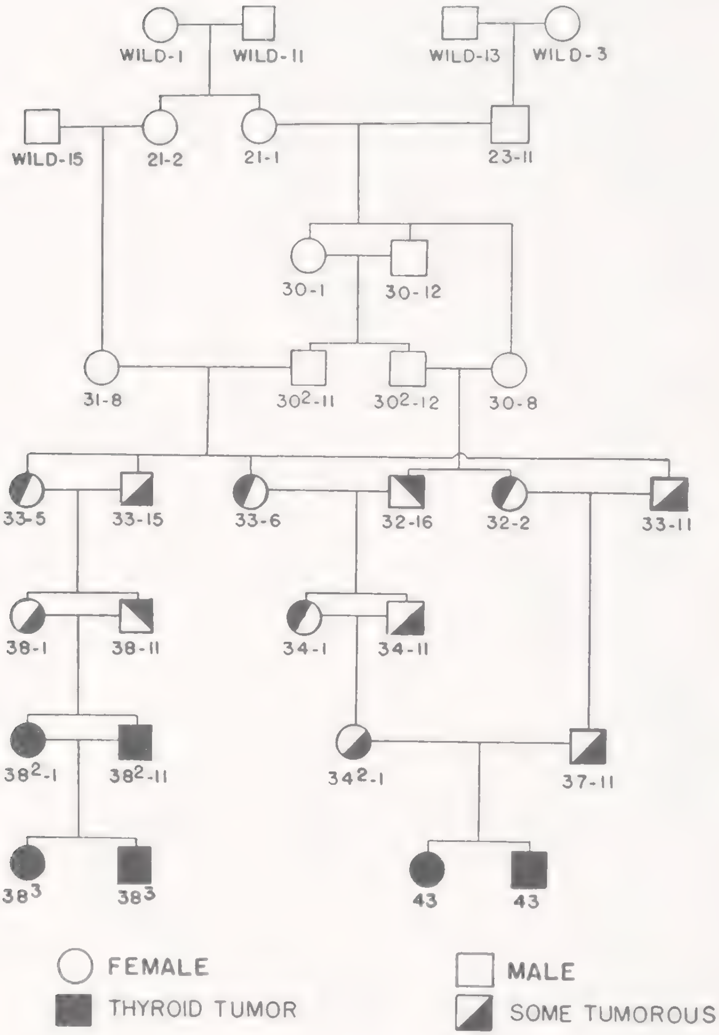

FIG. 11. The genetic history of two genetically different laboratory strains of Montezuma swordtails, $38^3$ and 43. They are the seventh-generation descendants of those collected in 1939 from Mexico. All the members of the seventh generation developed tumors, although tumors appeared in appreciable numbers in members of the third and fourth generations. The tumors develop earlier in the members of strain $38^3$ than in the members of strain 43. Note that the members of strain $38^3$ have been produced by three generations of brother-to-sister matings.

The mating procedure followed may be illustrated by tracing the history of tumorous fish $38^3$. In the mating of 33-5 and 33-15 the female and male were nontumorous but some of their siblings were tumorous. To diagram this mating the horizontal line connects the white areas of the circle and the square. In the next mating, below, a tumorous female (38-1) was mated to a nontumorous male (38-11). In this instance, the horizontal line connects the black area of the circle to the white area of the square.

normal follicles, very small follicles, nonfollicular cell masses, and a few cystic follicles.

*a. Progressive growth.* The progressive stages in the development of the thyroid tumor of the swordtail, *Xiphophorus montezumae*, are as follows: (1) Day-old fish have 20–30 thyroid follicles, lined by flat or low cuboidal epithelium, scattered in the stroma around the ventral aorta, but not in the gills. (2) The follicle cells increase in size and become high columnar. (3) The number of thyroid follicles increases; the blood capillaries in the region of the follicles become and remain engorged with red blood cells. (4) The epithelial cells of the new follicles simultaneously increase in number and size; some of the blood capillaries rupture, and individual red blood cells are found close to the follicular cells. (5) The follicle configuration becomes distorted. (6) The follicles disintegrate. (7) Tumorous growths develop, composed primarily of a mass of epithelial cells, microfollicles, and hemorrhages. (8) Muscles in the region of the ventral aorta are surrounded by tumorous afollicular epithelial cells. (9) Tumor cells destroy the deeper musculature, the gill

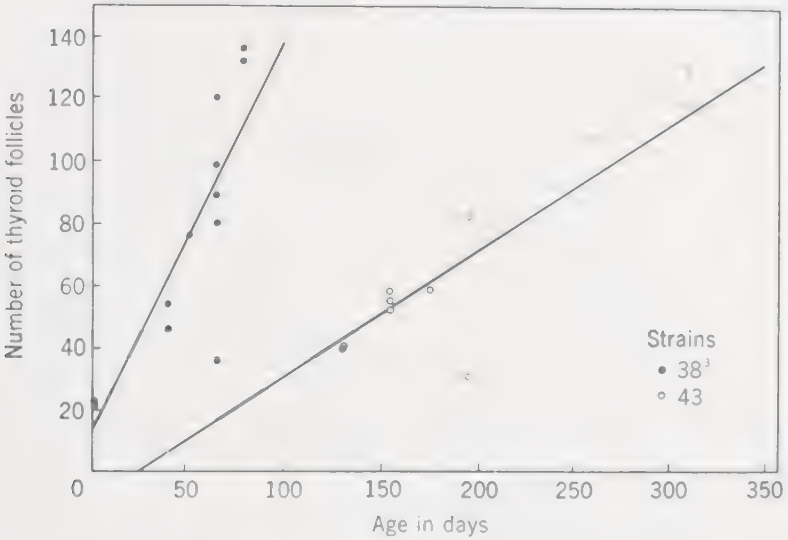

FIG. 12. The relationship between the number of thyroid follicles and the age of Montezuma swordtails. Right: white circles (○) represent laboratory-reared swordtails of strain 43. (Members of this strain develop tumors when 12 months old.) Note the slope of the regression line indicating that as the fish age there is a fairly rapid increase in the number of their thyroid follicles. Left: black circles (●) represent laboratory-reared swordtails of strain $38^3$. (Members of this strain develop tumors when 6 months old.) Note the steep slope of the regression line indicating that as the fish age there is a more rapid increase in the number of their thyroid follicles.

filaments, cartilage, and bone. (At this stage the thyroid tumor is visible externally.)

*b. Variations in sublines.* The number of thyroid follicles in relation to strain of fish was studied. At birth both normal and tumorous Montezuma swordtails have few thyroid follicles. As the fish develop, the number of follicles increases; but there is a distinct difference in their rate of multiplication in normal and tumor-susceptible swordtails. They increase slowly in wild swordtails, quite rapidly in laboratory swordtails of strain 43 (in which tumors develop at the end of a year), and extremely rapidly in members of strain 38 (which become tumorous 5 months after birth); see Fig. 12.

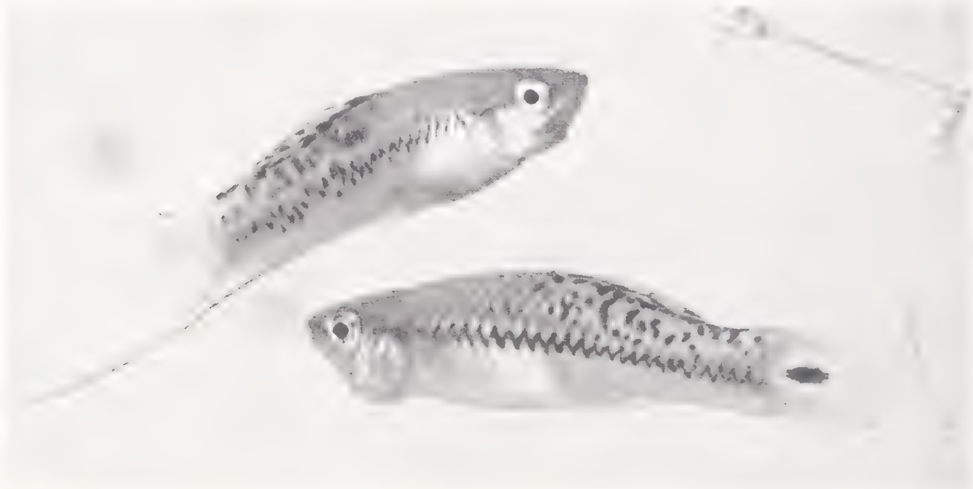

FIG. 13. Montezuma swordtails with thyroid tumors. The male on the left has a small visible nodule at the lower jaw. The female has a large tumor in the same region.

## 2. THYROID TUMORS IN THE KIDNEYS OF THE COMMON PLATYFISH

Several laboratory strains of platyfish, *Xiphophorus maculatus*, have been observed to have a high incidence of tumors involving the kidneys (Table VIII and Fig. 14). By morphological and physiological criteria these tumors were found to be thyroidal in nature. Radioautographs showed that the follicles of the kidney tumors are similar to those of the thyroid gland in their ability to bind inorganic iodine to protein. In addition to the kidneys, thyroidal tumor tissue was found in the heart, spleen, chorioid gland, and other areas of the body, but there was no direct evidence for metastases (Fig. 15). It is not known whether the tumors in the kidney and other sites are a result of migration from the thyroid itself, or whether they have differentiated *in situ* from undeterfied precursor cells. The tumors of the kidney are similar histologically to those of the thyroid, but are much more cystic. They, too, are able to invade and destroy muscle. The kidney tumor tissues may perforate the body wall and, in addition, may cause almost total destruction of the normal kidney tissue.

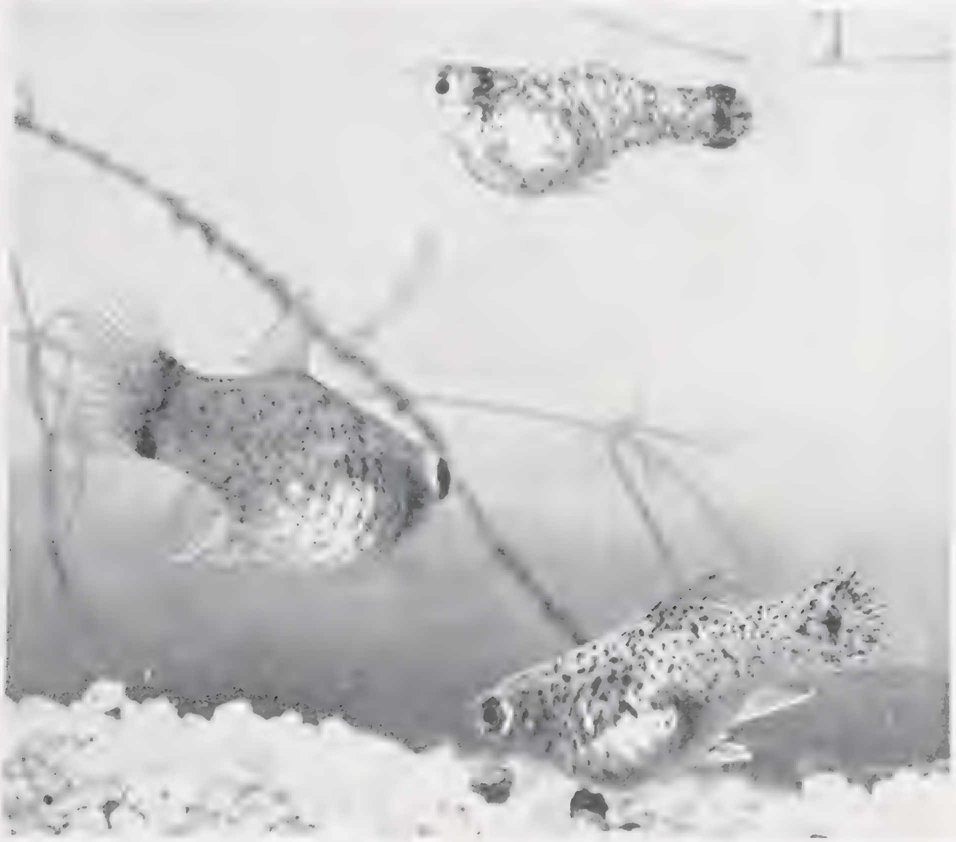

FIG. 14. Platyfish of strain BH that have thyroid tumors in their kidneys. The breakdown of kidney tissue is responsible for the visible edematous condition of the fish.

Since some strains of platyfish develop thyroidal tumors in the kidney and other strains do not, while kept under similar low iodine concentration of the laboratory aquarium water, it is inferred that there are genetic factors that mediate the differences in reaction.

*a. One subline resistant, one susceptible.* Platyfish from the Rio Jamapa that constitute our stocks "30" and "163" were obtained in 1939. Subline number "30" was isolated early and has been inbred for 20 generations by brother–sister matings. No thyroid or kidney tumors have appeared in its members. Subline "163" was not inbred by brother-sister matings until 7 generations back. Kidney tumors have often been found in the later generations of this stock (Table VIII).

As yet there is no evidence for genetic linkage between any of the many known color pattern genes and those which are probably responsible for various degrees of susceptibility to thyroid and kidney tumors.

TABLE VIII
HISTORIES OF PLATYFISH, *Xiphophorus maculatus*, SUSCEPTIBLE OR RESISTANT TO THYROID KIDNEY TUMORS

| I Strains | II Original Source of Strain | III Year Acquired for Laboratory | IV Generations | V Approximate Incidence of Kidney Tumors |
|---|---|---|---|---|
| *"Domesticated"* | | | | |
| BH | Everglades Aquatic Nurseries, Florida | 1947 | 7 | 27% + |
| Fury | Curt Kosswig, Istanbul, Turkey (since 1938) | 1950 | 10 | 55% + |
| Leopard | Behren's Aquarium, New York City | 1940 | 10 | Found |
| *"Wild"* | | | | |
| 163 | Río Jamapa, Mexico | 1939 | 7[a] | Found |
| 30 | Río Jamapa, Mexico | 1939 | 20[a] | None |
| Bp | Belize River, British Honduras | 1949 | 5 | 18% ± |
| Cp | Río Coatzacoalcos, Mexico | 1948 | 4 | Found |
| Gp | Río Grijalva, Mexico | 1952 | 2 | Found |
| Hp | Río Hondo, British Honduras | 1954 | Original | No data |

[a] Inbred by brother–sister matings.

## IX. ISOLATING MECHANISMS AND SPECIATION

### A. Psychological or Mating Behavior Patterns (see also Chap. XII: Part II)

It is significant that platyfish (*Xiphophorus maculatus*) hybridize with swordtails (*Xiphophorus helleri*) only under special conditions in the aquarium. Not a single hybrid between these species has ever been discovered in nature. Yet more than 7000 specimens of platyfish and swordtails have been collected under conditions of sympatricity in the Atlantic coastal freshwater areas of Mexico, British Honduras, and Guatemala. Moreover, because of extensive knowledge of the characteristics of hybrids based on laboratory experiments, there is no possibility that the collector has been unable to recognize the mongrel descendants of the backcrosses. With this information in mind Gordon (1947b) concluded that extrinsic isolating mechanisms alone are not critical at the present time in preventing an interchange in genes between the two species. He reviewed a number of possible intrinsic isolating mechanisms based on both morphological and physiological differences between the platyfish and swordtail and suggested that, above all others, psychological factors were probably the most important. The data on the distribution

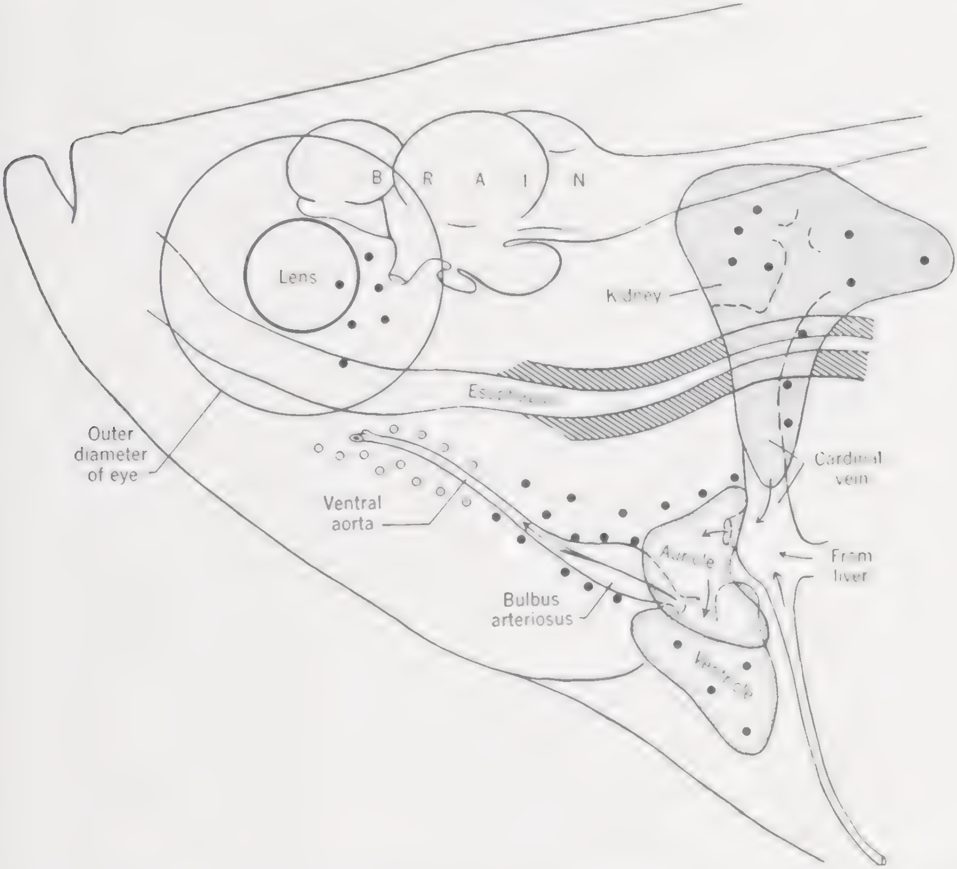

Fig. 15. Diagram of the anterior part of a platyfish showing the normal and typical distribution of thyroid follicles. In the eye the follicles are in the chorioid ayer but concentrated in the chorioid gland. In the heart and kidney the follicles re both inside and on their surfaces. Fish with thyroid tumors in the kidneys have typical thyroid follicles in all the areas shown and, in addition, in the spleen (not hown here). Some nontumorous fish have thyroid follicles in the kidneys as well as the normal thyroid area, but nowhere else.

of the two species in Mexico and Guatemala revealed that in 10 localities wordtails were found alone, in 5 localities platyfish were found alone, nd in 10 they were found living together. In the last areas they were often caught together in a single seine haul. A closer analysis of the data ndicated that when the swordtail and platyfish were taken from the ame river system, the swordtails were better represented in the head-waters and the platyfish in the lower reaches of the streams. To some degree, then, the species are isolated geographically and ecologically. Both species have the same breeding season and produce young throughout the year.

Clark, Aronson, and Gordon (1954) found that differences in sexual behavior between the platyfish and swordtail include qualitative and quantitative differences in courtship, precopulatory activity, duration time, and frequency of copulation. Just how the physiological and correlated morphological differences prevent interbreeding in mixed populations cannot be stated with certainty. It seems, however, that these items produce a whole pattern which contributes to the relative effectiveness of the mating situation. The complete mating pattern influences the interactions between the males and females and thereby builds up systems of attractions and repulsions.

Studies of the mating behavior patterns of the male platyfish (*Xiphophorus maculatus*), the swordtail (*Xiphophorus helleri*), their F$_1$, F$_2$, and backcross generation hybrids by Clark, Aronson, and Gordon (1954) have revealed some influence of heredity, but the genetic data were not of a kind that could be expressed in simple Mendelian terms.

Some of the precopulatory activities of xiphophorin fishes were found to be characteristic of the male platyfish (indicated by P) but not of the swordtail (indicated by X). Other traits were found to be represented in the reverse order. The frequency of a particular behavior in the F$_1$ (PX), F$_2$ (PX$^2$), and the two types of backcross male hybrids (PX-P, the hybrids produced by mating an F$_1$ female back to a platyfish; X-PX, the hybrids produced by mating a swordtail female to an F$_1$ male) would indicate the general manner of its inheritance (Fig. 16).

In a broad comparison of the frequencies of backing and nibbling on one hand with pecking and retiring on the other, an apparent pattern of inheritance is formed which becomes more apparent in the behaviors of the various backcross hybrids. Traits such as backing and nibbling, more or less restricted to the swordtail (X), are found more frequently in swordtail backcross hybrids (X-PX). Traits such as pecking and retiring, more or less restricted to the platyfish (P), are found more frequently in the platyfish backcross hybrids (PX-P).

Activities associated with precopulatory and copulatory activities have the following pattern of inheritance. The frequencies of gonopodial swinging are approximately similar in the platyfish (92.2%) and swordtails (78.0%) and their various hybrids. Except for an unexpected falling off in the frequency of this behavior among the F$_1$ hybrids (34.4%), all the other kinds of hybrids, the F$_2$ and the two backcross hybrids (PX-P and X-PX) are about the same (78.2%, 79.0%, 72.5%). The frequencies of gonopodial thrusts, too, are approximately similar in the platyfish (77.9%) and the swordtail (72.0%) and in their various hybrids: 46.2% in the $F_1$ hybrids, 66.3% in the $F_2$ hybrids, 48.1% in the platyfish backcross hybrids (PX-P), and 73.5% in the swordtail backcross hybrids (X-PX).

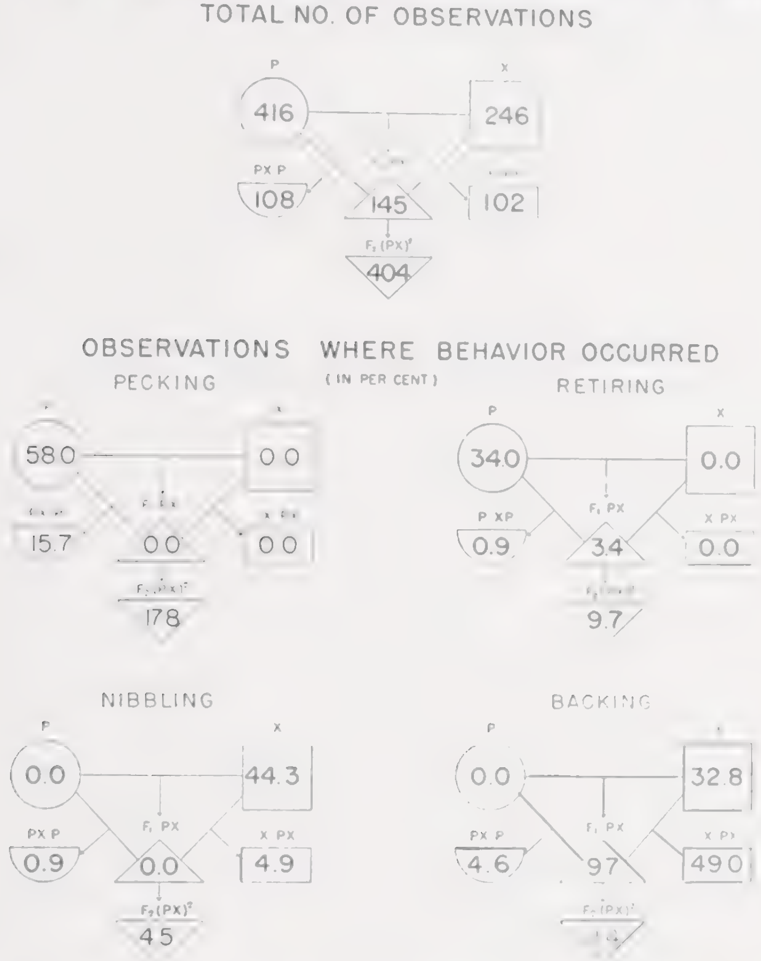

FIG. 16. Per cent of observations in which pecking, retiring, nibbling and backing behavior appeared in platyfish, swordtails, $F_1$, $F_2$, and backcross hybrids. P: platyfish, X: swordtail. $F_1$ PX: platyfish-swordtail $F_1$ hybrid. PX-P: Backcross hybrid obtained from the mating of an $F_1$ hybrid female with a platyfish male. X–PX: Backcross hybrid obtained from the mating of a swordtail female with an $F_1$ hybrid male. $F_2$ $(PX)^2$: The $F_2$ hybrids obtained by mating the $F_1$ brother to sister. (From Clark, Aronson and Gordon, 1954.)

The frequencies and duration of the behavioral elements directly associated with copulation and insemination in the platyfish, swordtails, and their various hybrids are evaluated as follows:

(1) The interval between the start of the observation and the first copulation is much longer in the platyfish (median = 5 minutes) than

in the swordtail (median = 1 minute). This is also short in the $F_1$ hybrids (median = 2 minutes), but in the $F_2$ hybrids it varies in duration from the shortest to the longest periods.

(2) The copulatory activities are higher in the platyfish (26.7% to 42.0%) than in the swordtail (13.4%). The frequency is slightly higher in the $F_1$ hybrids (29.0%), but lowest of all in the $F_2$ (8.4%) and in the two backcross generations: 7.4% in PX-P hybrids and 4.9% in the X-PX hybrids.

(3) The maximum number of copulations in a test period is higher in the platyfish (six) than in the swordtail (two). It is intermediate in the $F_1$ hybrids (five). In the $F_2$ hybrids the maximum number is two; in the platyfish backcross hybrids (PX-P) two; in swordtail backcross hybrids (X-PX) three.

(4) The duration of copulation in the platyfish is shorter (mean = 1.36 seconds) than in the swordtail (mean = 2.39 seconds). This difference when evaluated statistically appears to be significant. In the $F_2$ hybrids the average duration of copulation was very low (1.1 seconds) but here we were no longer dealing with a homogeneous group. Some males consistently copulated briefly, others copulated for a much longer time, and still others fell into one or more intermediate groups.

(5) The percentage of females inseminated during a test period following one or more copulations is higher in the platyfish (86.0%) than in the swordtail (39.4%); it is intermediate in the $F_1$ hybrids (64.3%), low in the $F_2$ (23.5%), and lowest in backcross generation hybrids (9.3% in PX-P, 0.0% in X-PX). In spite of the extremely low frequencies of successful insemination among the two groups of backcross hybrids, PX-P and X-PX, their precopulatory activities of swinging and thrusting were as high as those of the parental generation, indicating high levels of sexual arousal.

The isolating mechanism between the swordtail and the platyfish appears to depend, Clark, Aronson, and Gordon concluded, on the sum total of a variety of partially isolating factors. Each item alone is not sufficient to insure reproductive isolation, but acting together these factors so reduce the probability of hybridization that under natural conditions the isolation pattern as a whole seems to be completely effective. Extensive field data support this conclusion.

### B. Embryological Rates of Development

After making a thorough study of the embryological development of the platyfish and the swordtail, Tavolga (1949) compared their patterns of organogenesis with their laboratory produced $F_1$ and $F_2$ hybrids. He found only three points of difference, namely, time of initial pigment (micromelanophore) formation, rate of growth, and rate of caudal fin development.

In the platyfish micromelanophores were first visible at developmental stage 13 (4.8 days) in the outer retinal layer. In the swordtail they appeared first at stage 14 or 15 (5.0 to 5.5 days). In the $F_1$ and $F_2$ hybrids pigment appears at the 13th stage like that of the platyfish.

The growth rate of the platyfish embryo is somewhat slower than that of the swordtail so that the swordtail larvae attain a length one millimeter longer during a mean 21 day period of development. In the $F_1$ and $F_2$ hybrids the growth curves coincided with or slightly exceeded that of the swordtail.

The caudal fin begins to differentiate in the platyfish at stage 15, in the swordtail at stage 16; their $F_1$ and $F_2$ hybrids at stage 15.

From these data it would seem that the growth of some parts of the body in the developing hybrid resembles the platyfish while at the same time the rate of growth of the embryo as a whole resembles the swordtail.

In a series of papers Minamori (1950–1953) discovered many examples of physiological, geographical, and ecological isolation between five local races of the striated spinous loach, *Cobitis taenia striata*. The racial differences had a genetic basis. In hybridization experiments, aided by the induction of ovulation by injection of frog pituitary, he found that the male $F_1$ hybrids were mostly sterile. The females were on the whole less sterile. Nevertheless some of the larger eggs produced by the $F_1$ were successfully fertilized by sperm from members of the parental race and they developed to produce embryos, but similar eggs fertilized by sperm from $F_1$ males produced fewer viable embryos. Eggs produced by older females were more productive. Minamori indicated that eggs of cold water races are larger and more likely to be associated with viability of the embryos. This in turn is related to respiration rate. Embryos of small races respire more vigorously than those of larger ones. The oxygen consumption of the $F_1$ hybrids differed. A cold water female mated to a warm water male produced embryos that required less $O_2$ than their reciprocal $F_1$ hybrids.

After further testing the various races of *Cobitis taenia* by hybridization with the related mud loach, *Misgurnus anguillicaudatus*, Minamori summed up his results by saying that the degree of inviability in the generic hybrids is proportional to the differences in cell size between the parental species and races. Cell size was related to temperature adaptation; the varying reactions in the various types of hybrids paralleled those found by Moore (1946, 1949) for races of the frog, *Rana pipiens*.

## X. SUMMARY

### A. Evidence of Genetic Influences

In the study of genetic mechanisms for sex determination in vertebrate animals fishes are unique. In one species some races have the XX female, XY male system while others have the WY female, YY male mechanism. Some species of fishes have stable sex-determining mechanisms while others have indeterminate ones. The reports that fishes as a group are sexually labile have been exaggerated. These stem from two alleged instances of complete and functional sex reversal but these are questionable.

Genes are known in fishes which adversely affect their normal development. Some genes are lethal, killing the fish during embryonic development, others kill the fish before maturity. With regard to pigmentation, genes act in a variety of ways—some of them inhibit the elaboration of chromogens, others affect their enzyme systems.

In instances of abnormal growth in fishes, such as pigmented tumors, melanomas, and erythrophoromas, gene action plays the more important role. Study of melanomas of fishes genetically induced has revealed that a specific cell, the macromelanophore, is subject to pathological change by gene action.

Cataracts, tumors of the eye, and degeneration of the eyes that result in blindness are genetically determined in some fish species. The differences in flicker responses of closely related species of fishes may be attributed to genetic factors.

Many meristic traits that distinguish geographical races of fishes are inherited but here the environmental conditions strongly influence the final expression. The interplay of genetic and environmental factors make a precise analysis difficult.

In the analyses of the factors underlying success in transplantation of normal fins and of tumorous tissues, genetic correspondence between donor and host is of paramount importance. The genetic constitution of fishes may also determine whether or not they have greater tolerances towards high temperatures, to infectious diseases and to ultraviolet radiation.

In response to low iodine concentration in aquarium water some genetic strains of fishes develop thyroid tumors in the typical thyroid area; others develop thyroid tumors in the kidneys; while other strains are genetically resistant and their thyroids are normal.

The isolating mechanism that involves reproductive behavior has a genetic basis. This accounts for the lack of hybridization in nature between some closely related and sympatric species. When under laboratory conditions these are overridden, the genetic differences in the developmental rate of growth of embryos in each species often causes abnormalities in their hybrids, the most frequent being gonadal.

### B. Need for Inbred Stocks

Evidence has been presented that indicates that many physiological processes in fishes are conditioned by their genetic constitutions as well as by their external environment. From this evidence it would seem that physiologists should use genetically uniform fishes in their experiments. The use of standardized animals is as highly desirable as the use of pure chemical reagents and the maintenance of uniform environmental conditions. At present most of the fishes used by physiologists are extremely heterogeneous since they are obtained from dealers, fish hatcheries, or are taken from natural sources. Experimental animals that are obtained from their natural waters may appear to be more uniform but competent tests reveal that the genetic variability of wild fishes is great. Stocks of the same species utilized by two groups of physiologists in their experiments may yield results that differ widely even though the experiments may have been conducted by similar techniques and procedures. To decrease the genetic variability some degree of inbreeding is desirable. For some critical experiments only the use of highly inbred fishes may insure valid and verifiable results.

Highly inbred strains are known in possibly three species of laboratory fish. The platyfish, *Xiphophorus maculatus*, has been bred by brother to sister matings for twenty generations and the swordtail, *X. helleri*, for fourteen generations at the Genetics Laboratory of the New York Zoological Society at the American Museum of Natural History. The guppy, *Lebistes reticulatus*, in a number of stocks for various color genes, has been maintained in isolated mass cultures for over a quarter of a century by the Haskins Laboratory of New York City. Brood stocks of these rare and valuable fishes most likely are available to competent and responsible workers everywhere in the world.

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# AUTHOR INDEX

Numbers in italics indicate the pages on which the references are listed.

A

Abel, R., 45, *106*
Abood, L. G., 303, *304*
Abramson, H. A., 303, *304*
Abramowitz, A. A., 14, 100, *104*, *105*
Achtenberg, H., 313, *321*
Adams, L. A., 470, *500*
Adanson, M., 323, *340*
Addens, J. L., 13, *105*
Adrian, E. D., 45, 78, *105*, 141, 142, 143, *150*, 193, 194, *207*, 238, *265*
Agersborg, H. P. K., 103, *105*
Aida, T., 433, 434, 435, 448, 449, 469, *493*, *494*
Akabane, T., 347, *364*
Akert, K., 22, 23, 25, 26, 27, *105*
Akita, U. K., 309, 310, *318*
Albe-Fessard, D., 50, *105*, 330, 331, 332, 333, 336, 337, 338, *340*, *341*
Alcock, A., 356, *364*
Alford, R. S., 315, *320*
Allen, F., 132, *150*
Allen, W. F., 126, *150*
Allison, A. C., 193, *207*
Alm, G., 471, *494*
Almeida, D. F., 334, *341*
Altamirano, M., 330, 332, 333, 334, 336, 339, *341*
Anderson, B. G., 420, *428*
Apter, J. T., 22, *105*
Arey, L. B., 134, 135, *150*
Arnaudet, A., 45, *105*, *106*
Aronowitz, O., 439, *497*
Aronsohn, E., 196, *207*
Aronson, L. R., 230, 262, *265*, *266*, 273, 274, 278, 281, 282, 283, 285, 287, 288, 289, 290, 291, 292, 293, 295, 296, *296*, *297*, *299*, 442, 443, 473, 488, 489, *494*
Atz, J. W., 284, *301*
Auger, D., 334, *341*
Augustinsson, K.-B., 312, *318*

B

Babak, E., 78, *105*
Bachrach, E., 50, *105*
Baerends, G. P., 230, 234, 235, 242, 248, 249, 253, 254, 255, 256, 258, 259, 261, 262, 264, *265*, 278, 279, 280, 282, 293, 294, 295, *296*
Baerends-van Roon, J. M., 230, 235, 242, 253, 256, 258, *265*, 278, 279, 280, 294, 295, *296*
Baggerman, B., 304, *304*
Baglioni, S., 196, *207*, 312, 313, *318*
Bailey, R. M., 442, 472, *494*, *499*
Baker, K. F., 449, 479, *494*, *497*
Baldwin, F. M., 284, 286, *296*
Ball, E. G., 309, *318*, *322*
Ballowitz, E., 97, *105*
Bandt, H. J., 420, 426, *427*
Barbour, H. G., 99, *114*
Baron, J., 131, 133, *150*
Baron, M. O., 303, *304*
Barraud, E. M., 296, *296*
Barron, D. H., 101, *105*
Bastock, M., 235, 247, *265*
Baudelot, M. E., 6, 16, 17, 36, 43, 44, *105*
Bauer, V., 55, 64, 73, 81, *105*
Bayliss, L. E., 136, 138, 139, 149, *150*
Beach, F. A., 273, 276, 283, 285, 286, 296, *296*
Beadle, G. W., 432, *494*
Bechtereff, W. V., 213, *224*
Beebe, W., 348, 354, *364*
Beer, T., 131, *150*
Belding, D. L., 276, 282, 294, *296*
Bellamy, A. W., 435, *494*
Benditt, E. C., 310, *318*
Benjamins, C. E., 177, *182*
Benzer, P., 468, *497*
Berg, O., 442, 443, 479, 480, *494*
Beritoff, J., 85, *105*
Bernstein, J., 332, 335, 336, *341*

Bert, P., 99, *105*
Bertelsen, E., 355, 360, *364*
Berwein, M., 10, *105*
Bethe, A., 6, 17, 18, 36, 43, 53, 72, 73, *105, 106*
Beyer, L., 282, *296*
Bhimachar, B. S., 3, *106*
Bickel, A., 57, 66, 68, 74, 75, *106*
Biedermann, W., 325, 332, *341*
Bigelow, H. B., 177, *182,* 338, 339, *341*
Billings, V. N., 283, 285, 287, 288, *301*
Binet, L., 45, 50, *105, 106*
Black, E. C., 310, *318, 319,* 412, *429*
Black, V. S., 308, 309, 313, *318,* 412, *429*
Blackburn, M., 371, *383*
Blaue, J., 193, *207*
Bliss, A. F., 138, *150*
Blume, W., 50, *106*
Bock, F., 285, *296*
Bodian, D., 3, *106*
Boeseman, M., 261, *268*
Bohr, C., 92, *106,* 312, *318*
Boie, H. J., *224*
Boll, F., 135, *150*
Bonnet, V., 45, *106*
Borne, R., 285, 286, 289, *300*
Borodin, N. A., 409, *427*
Braude, E. A., 372, *384*
Brauer, A., 348, 356, *364*
Braus, H., 48, *106*
Bray, A. W. L., 99, *106*
Breder, C. M., Jr., 14, 53, *106,* 147, *152,* 230, *265,* 274, 276, 277, 279, 281, 283, 284, 294, 295, *296, 297,* 330, *341,* 466, 467, *494*
Breider, H., 445, *494*
Bresse, G., 276, *297*
Bretschneider, L. H., 291, *297*
Brett, J. R., 201, *207,* 407, 408, 409, *427, 428, 429*
Bridge, T. W., 314, 315, *318*
Bridges, W., 147, *150*
Brinley, F. J., 103, *106*
Brock, L. G., 330, 334, *341*
Brown, F. A., 311, 312, *318*
Brown, G., 78, *106*
Brown, M. V., 334, 336, 337, *341, 343*
Brouwer, R., 230, 234, 249, 258, 259, 264, *265,* 282, 293, *296*
Browness, J. M., *150*
Bucher, V., 22, 24, *109*
Bürgi, S., 22, 24, *109*
Burdick, G. E., 413, 415, 420, 424, *428*
Bull, H. O., 176, *182,* 215, 216, 217, 221, 222, 224, *224, 225, 227*
Bullough, W. S., 275, *297*
Burckhardt, C. R., 355, *364*
Burden, W. D., 197, *207*
Burger, J. W., 275, 284, *297*
Burkenroad, M. D., 315, *318*
Burne, R. H., 190, *207*
Burrows, H., 286, *297*
Buser, P., 32, 33, *106, 107,* 143, *150*
Busnel, R. G., 369, 370, *383, 384*
Buytendijk, F. J. J., 45, 78, *105,* 238, *265*

C

Calkins, E., 455, *495*
Cameron, G., 453, *497*
Cardot, H., 45, 49, *105, 106, 107*
Carlson, A. J., 3, *107*
Carpenter, K. E., 425, *428*
Carranza, J., 276, 294, 295, *297*
Cason, H., 212, *225*
Chagas, C., 333, 334, 338, *340, 341*
Chagnon, E. C., 177, 178, 180, 181, *184,* 220, *226,* 314, *320*
Chauchard, A., 7, 18, 19, 24, *107*
Chauchard, B., 7, 18, 19, 24, *107*
Chavin, W., 439, *494*
Chen, J., 160, 162, *184*
Cheyne, H., 412, 414, *430*
Ciabatti, O., 99, *107*
Claff, C. L., 311, 317, *321*
Clark, E., 35, *115,* 130, *153,* 230, *266,* 273, 282, 292, 293, *296, 297,* 442, 443, 473, 488, 489, *494*
Clark, E. E., 475, *498*
Clarke, G. L., 121, *150*
Clarke, R., 355, *364*
Clawson, G. H., 407, 409, *429*
Coates, C. W., 279, 294, *297,* 324, 330, 331, 332, 333, 334, 336, 337, 339, *341, 343*
Coghill, G. E., 103, *107*
Cohen, H., 287, *297*
Cole, A. E., 418, *428*
Collias, N. E., 277, *297*

Collins, F. D., 136, 137, *150*
Cooper, O., 309, *318, 322*
Copeland, D. E., 309, 311, 312, *318*
Corso, F., 36, *107*
Couceiro, A., 331, 334, *340, 341*
Cox, R. T., 330, 331, 334, 337, *341*
Craig-Bennett, A., 275, *297*
Craig, W., 236, 248, *266*
Craigie, E. H., 206, *207*
Crane, J., 354, 360, *364*
Crane, S. C., 371, 382, *384*
Craw, H., 55, *107*
Crescitelli, F., 137, *150*
Crosby, E. C., 3, 4, 16, 36, 38, 40, 42, 50, *111*
Crozier, W. J., 139, 140, 149, *151*, 465, 466, *494*
Cunningham, J. T., 315, *318*
Curtis, B., 230, 253, 256, *267*, 277, 278, 279, 281, 282, 283, *300*

D

Dahlgren, U., 324, 325, 329, 330, 338, *341, 342*, 354, *365*
Daitch, B., 293, 295, *301*
Dale, H. H., 100, *107*
Damant, G. C. C., 308, *319*
Dammerman, K. W., 12, *107*
Dartnall, H. J. A., 137, *150, 151*
Darwin, C., 339, *342*
Das, K., 275, *298*
Das Gupta, B., 275, *298*
Daugherty, F. M., Jr., 424, *428*
Davies, R. E., 309, *318*
Davis, H. S., 476, *494*
Davison, R. C., 414, *428*
Davson, H., 126, *151*, 336, *342*
de Burlet, H. M., 173, 181, *182*
Dean, H. F., 413, 415, *428*
Degens, P. N., 427, *428*
Denker, A., 177, *182, 225*
Denny, M., 43, *107*
Desmoulins, A., 6, *107*
Detwiler, S. R., 123, 127, 132, 134, *151*
Devlaminck, F., 421, 427, *429, 430*
Dickinson, W. H., 36, *107*
Diesselhorst, G., 176, 180, *183*
Dieuzeide, R., 383, *384*
Dijkgraaf, S., (Dykgraaf, S.), 17, 20, 21, 30, 35, 39, 41, 74, *107, 108*, 156, 158, 159, 160, 163, 176, 177, 180, 181, *183, 186*, 195, 196, *207*, 222, 223, *225, 228*, 282, *297*, 314, 315, *318, 319*
Dirken, M. N. J., 43, 46, *118*
Ditlevsen, E., 438, 448, *501*
Dobrin, M. B., 315, *319*
Dotterweich, H., 165, *183*
Douard, T., 383, *384*
Doudoroff, P., 406, 407, 408, 409, 410, 411, 412, 418, 420, 424, 426, 427, *428, 430*
Duane, T. D., 151, *151*
Du Bois-Reymond, E., 332, 338, 340, *342*
Dufossé, M., 315, *319*
Duke-Elder, S., 126, *151*
Duncan, M. C., 273, 293, 295, *297, 301*
Dussadier, M., 33, *107*
Duyvené de Wit, J. J., 291, 295, *297*
Dykgraaf, S., *see* Dijkgraaf, S.

E

Earnest, D., 414, *430*
Eberhard, K., 64, *108*, 239, *266*
Eberhardt, K., 448, *495*
Eccles, R. M., 330, 334, *341*
Edgar, M., 479, 480, *494*
Edgerton, H., 53, *106*
Edinger, L., 40, *108*
Egami, N., 274, 291, *297*, 472, 473, *495*
Eggert, B., 279, 282, 291, *298*
Eimer, T., 173, *183*
Ellis, M. M., 324, *342*, 405, 406, 410, 418, *428*
Embody, G. C., 475, 476, *495, 497*
Emling, J. W., 315, *320*
Enami, M., 393, *401*
Engelhorn, O. R., 416, *429*
Erdl, M. P., 323, 330, *342*
Ermin, R., 449, 455, *495*
Escher-Desrivières, J., 140, *151*
Essenberg, J. M., 446, *495*
Evans, H. E., 3, *108*
Evans, H. M., 195, *207*, 290, *298*, 308, *319*
Evans, L. T., 303, *304*
Eversole, W. J., 287, *298*
Ewart, J. C., 328, *342*

F

Fabricius, E., 254, *266*, 274, 275, 276, 277, 282, 294, 295, *298*
Fabricius, M., 64, *108*, 239, *266*
Fänge, R., 92, *108*, 305, 306, 308, 309, 310, 311, 312, 313, *318*, *319*
Farkas, B., 176, *183*, *225*
Fatt, P., 334, 335, 336, *342*
Feldberg, W., 335, *342*
Ferrier, D., 6, 7, 17, *108*
Fessard, A., 334, 335, *341*, *342*
Fish, M. P., 282, *298*, 315, 316, *319*
Fisher, P., 220, *225*
Fitzpatrick, T. B., 455, *495*
Florey, E., 49, *108*, 303, *304*
Florkin, M., 136, 137, *151*
Flourens, P., 6, 43, *108*
Fontaine, M., 369, 370, *383*, *384*
Fox, D. L., 367, 368, 371, 373, 375, 382, *384*, *385*
Franz, G., 312, 314, *319*
Franz, V., 40, *108*
Franzisket, L., 260, *266*
Fraser, A. C., 435, 436, 469, *495*, *497*
Frédéricq, L., 55, *108*
Freire, J. R. C., 334, *341*
Friess, E., 446, *495*
Friedländer, B., 66, *108*, 236, *266*
Friedman, B., 443, *495*
Friedman, M., 291, *298*
Friedrich-Freska, H., 164, *183*
Fritsch, G., 325, 329, 330, *342*
Froloff, J. P., 7, *108*, 176, 177, *183*, 216, *225*
Fry, F. E. J., 405, 407, 409, 412, *429*, 475, *495*
Fuchs, R. F., 388, *401*
Fuji, K., 331, *342*
Fuller, A. C., 412, *430*
Fulton, J. F., 3, *108*

G

Gabriel, M. L., 468, 469, *495*
Gage, S. P., 288, *298*
Galstoff, P. S., 420, *428*
Garrett, J. T., 424, *428*
Garrey, W. E., 410, *429*
Gaufin, A. R., 406, *429*
Gawrilenko, A., 189, *207*
Gaylord, H. R., 478, *495*
Geddes, P., 433, *495*
Genguly, D. N., 129, *152*
Geronimus, L. H., 303, *304*
Gettner, H. H., 303, *304*
Gibson, M. B., 470, 475, *495*
Giersberg, H., 99, *108*
Gierse, A., 356, *365*
Gill, T., 282, *298*
Gilman, A., 100, *108*
Giovanella, B., 475, *495*
Gladstone, R. J., *151*
Glover, M., 383, *384*
Göz, H., 199, 200, *207*, 221, *225*
Gohar, H. A. F., 262, *266*
Goldschmidt, R., 432, *495*
Golovinskaya, K., 470, *495*
Goodman, L. S., 100, *108*
Goodrich, E. S., 48, *108*, 157, 158, *183*, 307, *319*
Goodrich, H. B., 448, 449, 450, 469, 473, 478, *495*, *496*
Goodwin, T. W., 373, 375, *384*
Gorbman, A., 478, 479, *494*, *496*
Gordon, M., 230, *266*, 273, 291, 292, 293, *297*, *301*, 371, *384*, 434, 435, 436, 439, 440, 442, 443, 445, 447, 448, 449, 450, 452, 453, 455, 458, 459, 460, 461, 462, 463, 464, 465, 468, 469, 472, 473, 474, 475, 477, 478, 479, 480, 486, 488, 489, *494*, *495*, *496*, *497*, *498*, *499*
Gota, T., 370, *384*
Gotch, F., 332, 337, 338, *342*
Gourevitch, A., 369, *384*
Gowen, J. W., 477, *497*
Graham, H. T., 3, 50, *108*
Graham, J. M., 405, 414, *429*
Graham Brown, T., 236, *266*
Grand, C. G., 453, *497*
Grangaud, R., 383, *384*
Granit, R., 138, 139, 140, 141, 149, *151*
Gray, J., 53, 56, 57, 63, 69, 70, 71, 73, 75, 80, 85, 97, 99, 100, 104, *108*, *109*, 236, *266*
Greeley, J. R., 294, *298*
Green, A. A., 309, 310, *319*
Greenberg, B., 230, 261, *266*, 277, *298*
Greenberg, S. S., 455, 464, 475, *497*
Greene, C. W., 315, *319*, 347, 360, 361, 362, 363, *365*
Greene, H. H., 347, 362, 363, *365*
Gresser, E. B., 466, *494*

Griffin, D. R., 316, *319*
Grinnell, S. W., 310, *319*
Groen, J. J., 171, *183*
Grøntved, J., 360, *364*
Grundfest, H., 122, 139, 149, *151*, 330, 332, 333, 334, 336, 339, *341*
Gustafson, K. J., 277, 282, 294, 295, *298*
Guyénot, E., 308, 314, *319*

H

Haddon, H. C., 314, 315, *318*
Hämmerling, J., 445, *497*
Haempel, O., 177, *183*
Hafen, G., 177, *183*, *225*
Haffner, R. E., 348, *365*
Hagman, N., 315, *319*
Haldane, J. A., 310, *319*
Haldane, J. B. S., 432, 442, *497*
Hale, E. B., 304, *304*
Hall, F. G., 309, 313, *319*
Haller von Hallerstein, V., 195, *209*
Hama, T., 370, *384*
Hamilton, W. F., 140, *151*
Handrick, K., 356, *365*
Haneda, Y., 347, 349, 350, 352, 353, 356, *365*, *366*
Hardenburg, J. D. F., 315, *319*
Hanzawa, S., 64, *116*
Harlow, H. F., *226*
Harms, J. W., 354, *365*
Harnisch, O., 418, *429*
Harris, E. F., 413, 415, *428*
Harris, J. E., 53, 101, 102, *109*
Harrison, R. W., 469, *495*
Hart, J. S., 405, 407, 409, *429*, 475, *497*
Hart, W. B., 420, *428*
Hartline, H. K., 142, 143, *151*
Harvey, E. N., 346, 347, 348, 352, 355, 356, 359, *365*
Haskins, C. P., 261, *266*, 279, 293, *298*, 448, *497*
Haskins, E. F., 261, *266*, 279, 293, *298*, 448, *497*
Hasler, A. D., 125, *153*, 202, 203, 204, 205, 206, *207*, *208*, *209*, 221, 224, *226*, *228*, 284, *298*
Hayes, C., 245, *266*
Hayes, J. S., 245, *266*
Hayford, C. O., 475, 476, *495*, *497*
Healey, E. G., 98, 99, 101, *109*, 392, *401*
Hebb, D. O., 53, *109*, 273, *298*
Hecht, S., 132, 138, 140, *151*
Heinroth, O., 231, *266*
Held, E. E., 474, *497*
Helfer, H., 418, *429*
Hellauer, H. F., 49, *116*
Herbert, D. W. M., 423, *429*
Herrick, C. J., 3, 4, 40, *109*, 195, 196, *208*, 288, 289, 290, *298*
Herter, K., 7, *109*, *220*, *226*
Hess, E. H., 254, *266*
Hess, W. R., 22, 24, *109*
Heuts, M. J., 467, 470, 471, *497*
Hiatt, R. W., 201, *208*
Hickling, C. F., 350, 355, 356, *365*
Hilgard, E. R., 204, *208*, 213, *226*
Hinde, R. A., 245, 249, 250, 260, *266*
Hineline, G. M. W., 140, *151*
Hoagland, H., 40, 43, *109*, 160, 163, *183*
Hoar, W. S., 15, *109*, 287, 288, *298*
Hodgkin, A. L., 335, 336, *341*
Hoessly, G. F., 22, *109*
Hofer, B., 158, 160, *183*
Hogben, L. T., *401*
Holz-Tucker, A. M., 287, 291, *296*
Holzapfel, M., 248, *266*
Hooker, D., 52, 74, 101, 103, *109*, *110*
Hoover, E. E., 274, *298*
Hora, S., 275, *298*
Horio, G., *226*
Hosch, L., 10, *110*
Hubbard, H. F., 274, *298*
Hubbs, C. L., 382, *384*, 443, 445, 468, 472, *497*, *498*
Hubbs, L. C., 443, 445, *498*
Huber, G. C., 3, 4, 16, 36, 38, 40, 42, 50, *111*
Huet, M., 421, *430*
Humm, D. G., 475, *498*
Huntsman, A. G., 410, *429*
Husain, A., 275, *298*
Hyde, I. H., 44, *110*
Hyman, L. H., 468, *498*

I

Ikeda, K., 285, *299*
Irving, L., 310, *318*, *319*, *321*
Isaka, S., 369, 370, *384*

Ishida, S., 369, *384*
Ishihara, M., 433, 448, *498*
Ishikawa, C., 433, *498*

J

Jacobs, W., 309, 312, *319*
Jagodowski, K. P., 193, *208*
Jakowska, S., 462, 463, *499*
Jahnel, J., 465, *498*
Janzen, W., 8, 9, *110*
Jaski, C. J., 282, *299*
Johann, L., 347, 355, *365*
Johnson, M. W., 315, *319*
Jones, F. R. Harden, 307, 308, 311, 312, 314, 317, *320*
Jones, J. R. E., 425, 426, *429*
Jones, J. W., 285, 287, 294, 295, 299
Josephson, N. D., 469, *495*
Jucker, E., 372, *384*
Jullien, A., 93, 94, 95, 96, 97, 104, *110*, *111*

K

Kadow, P., 293, *299*
Kahmann, H., 133, *151*
Kaiser, P., 286, *299*
Kalischer, O., 213, *226*
Kallman, K., 474, *498*
Kamphuis, A. H., 427, *428*
Kamrin, R. P., 289, 292, *297*, *299*
Kann, S., 309, *321*
Kappers, C. U. A., 3, 4, 16, 36, 38, 40, 42, 50, *111*
Karamian, A. I., 39, 41, *111*, *226*
Karrer, P., 372, *384*
Karsten, A., 423, *429*
Kato, J., 369, *384*
Kato, K., 354, *365*
Katsuki, Y., 160, 162, *183*, *184*
Katz, B., 334, 335, 336, *342*
Katz, M., 406, 410, 411, 412, 418, 424, 426, 427, *428*, *429*
Kawamoto, N. Y., 139, *151*
Kazansky, B. N., 274, *299*
Keenleyside, M. H. A., 263, *266*
Keller, D. L., 303, *304*
Kelsey, A. S., 315, *319*
Kennedy, J. S., 238, *267*, 273, *299*
Kepner, W. A., 327, *342*
Keynes, R. D., 328, 330, 331, 332, 333, 334, 336, 339, *341*, *342*
Khalil, F., 313, *320*
Khan, H., 275, *299*
King, G. M., 285, 287, 294, 295, *299*
King, J. C., 477, *498*
Kirpichnikov, V. S., 449, 469, *498*
Kirsche, W., 52, *111*
Kishitani, T., 350, *365*
Kistiakowsky, G. B., 188, *208*
Kleerekoper, H., 177, 178, 180, 181, *184*, 220, *226* 314, *320*
Klenk, F., 197, *208*
Knudsen, V. O., 315, *320*
Koch, H., 308, *320*
Koehler, O., 234, *267*
Kohsen, A., 245, *266*
Koike, I., 331, *342*
Kokas, E. V., 314, *320*
Kommer, J. D., 427, *428*
Konishi, J., 139, *151*
Konorski, J., 213, *226*
Kopac, M. J., 455, 475, *497*
Koppányi, T., 51, 52, *111*, *113*
Kornetsky, C., 303, *304*
Kortlandt, A., 247, 248, *267*
Koshtojanz, C. S., 314, *320*
Kosswig, C., 435, 445, 447, 448, 458, 486, *498*
Kouliabko, A., 44, *111*
Krause, A. C., 124, *151*
Krausse, A., 177, *184*
Krinner, M., 197, *208*, 221, *226*
Kritzler, H., 382, *384*
Krumholz, L. A., 294, *299*
Kubo, I., 173, *184*
Kudrajaschoff, B. A., 305, *320*
Kühne, W., 135, *151*
Kuiper, K., 312, 314, *320*
Kumakura, S., 8, 10, *111*
Kumpf, K. F., 283, 285, 286, 287, 288, *300*
Kushibiki, K., 370, *384*
Kuwabara, S., 349, 353, *366*
Kyle, H. M., 272, *299*, 314, *320*, 433, *498*

L

Lage, H. V., 336, *342*
Lagler, K. F., 442, 472, *494*, *499*
Langham, M., 124, *152*
Langley, J. N., 91, *111*
Laroze, A., 426, *429*

Laskowski, W., 287, 295, *299*
Laurent, P., 104, *111*
Lavenda, N., 446, *499*
Le Mare, D. W., 56, 64, 65, 73, *111*, *118*, 237, 238, 239, *267*, *269*
LeClerc, E., 421, 427, *429*, *430*
Leghissa, S., 102, 103, *111*
Lehrman, D., 230, 252, *267*, 273, 280, 289, *299*
Leigh-Sharpe, W. H., 291, *299*
Leiner, G., 309, *320*
Leiner, M., 230, *267*, 281, 282, *299*, 309, *320*
Lenhart, C. H., 478, *499*
Lerner, A. B., 455, *495*
Levine, M., 464, *499*
Li, Min-Hsin, 284, 286, *296*
Liddell, H. S., 212, *226*
Liermann, K., 190, 191, 192, 193, *208*
Lindeman, V. F., 126, *152*
Lindsey, C. C., 469, *499*
Lindworth, A., 295, *298*
Lipschuetz, M., 413, 415, 424, *428*
Lissmann, H. W., 56, 73, 74, 76, 79, 80, 81, 82, 85, *111*, *112*, 230, 237, 239, 254, 261, *267*, 324, 330, 332, 339, *343*
Livanov, M. N., 314, *322*
Loeb, J., 6, 18, 36, 43, *112*, 407, *429*
Longwell, J., 423, *429*
Lorenz, K., 231, 232, 233, 238, 248, 256, 261, 264, *267*
Lowenstein, O., 133, *152*, 161, 170, 171, 172, 173, 174, 175, 181, 182, *183*, *184*, 315, *320*
Lowndes, A. G., 307, 308, *320*
Lowrey, A., 328, *343*
Loye, D. P., 315, *320*
Lucké, B., 478, *500*
Ludwig, C., 193, 194, *207*
Lundberg, A., 335, *343*
Lutz, B. R., 45, *112*
Lyon, E. T., 174, *184*
Lythgoe, R. J., 136, 138, 139, 149, *150*

**M**

McDonald, H. E., 177, 178, *184*, 220, *226*
McDougal, R. C., 132, *152*
McGavock, A. M., 412, 415, *430*
MacGinitie, G. E., 278, 279, *299*
MacKinnon, D., 201, *207*, 287, *298*
McLennan, H., 303, *304*
McNally, W. J., 174, *184*
McNamara, F., 274, *299*
MacNaughton, I. P. J., 174, 178, *184*
MacNichol, E. F., Jr., 143, *151*
Magendie, S., 6, *112*
Maier, H. N., 177, *184*
Maksutow, J. C., 305, *320*
Mangold, E., 356, 357, *366*
Manning, F. B., 174, 177, *184*
Marcus, H. C., 412, *430*
Marcus, T. R., 455, 474, *499*
Marine, D., 478, *499*
Marquis, R. G., 204, *208*, 213, *226*
Marsh, M. C., 478, *495*
Marshall, N. B., 308, 314, 317, *320*
Martins-Ferreira, H., 50, *105*, 328, 332, 333, 336, 337, *340*, *341*, *342*
Massonet, R., 383, *384*
Mast, S. O., 141, *152*
Mather, K., 470, *499*
Mathews, S. A., 275, *299*
Matthews, D. C., 201, *208*
Matthews, R., 142, *150*
Matthey, R., 443, *499*
Maxwell, S. S., 171, 173, 174, *184*
Mazzi, V., 15, *112*
Meader, R. G., 6, 10, 12, 27, *112*, 131, 132, *152*, 288, *299*
Medawar, P. B. 446, *499*
Medlen, A., 275, *299*
Meesters, A., 311, 312, *320*
Mercer, R. N., 448, *495*
Merkens, J. C. 423, *429*
Merker, E., *152*
Merriman, D., 275, *300*
Metcalf, H. E., 165, *184*
Meyer, H., 445, *499*
Meyer, R. K., 284, *298*
Mikhaïloff, S., 213, *226*
Millott, N., 368, *384*
Mills, S. M., 100, *112*
Minamori, S., 491, *499*
Mintz, E. U. 132, 140, *151*
Mitchell, H. K., 432, *500*
Mittelstaedt, H., 30, *118*
Mobius, K. A., 315, *320*
Moehres, F. P., 314, *321*
Moller-Racke, I., 30, *117*
Moncrieff, R. W., 118, *208*

Mookerjee, H., 275, *298*
Mookerjee, H. K., 129, *152*, 275, *299*, *300*
Mookerji, P. S., 129, *152*
Moore, G. A., 132, *152*
Moore, J. A., 491, *499*
Moore, W. G., 413, *430*
Moorhouse, V. H. K., 176, *185*, *226*
Moreau, F. A., 313, *321*
Morris, D., 230, 235, 247, *265*, *267*, 277, 295, *300*
Morrison, P., 310, *318*
Morton, R. A., *150*, 383, *384*
Mosher, C., 278, *300*
Mowbray, W. H., 315, *319*
Moynihan, M., 235, 247, *265*
Müller, E., 48, *112*
Müller, J., 315, *321*
Mundt, G. H., 135, *150*
Murray, H. D., 121, 135, *152*
Murray, R. W., 168, *185*
Muskens, L. J. J., 18, *112*
Mygind, S. H., 173, *185*

N

Nachmansohn, D., 334, 336, *343*
Nagel, P., 311, 312, *320*
Nagel, W. A., 6, *112*, 196, *208*
Naughton, J. J., 201, *208*
Nayar, D. K., 147, *152*
Needham, P. R., 294, *300*
Neurath, H., 204, *208*, 215, 221, *226*
Nichols, R., 473, *495*
Nicol, J. A. C., 91, 92, 93, 97, *112*, 363, *366*
Nigrelli, R. F., 462, 463, 467, 478, 479, *494*, *499*
Noble, G. K., 10, *112*, 200, *208*, 230, 253, 256, *267*, 277, 278, 279, 281, 282, 283, 285, 286, 287, 288, 289, 290, 295, *300*
Nolte, W., 8, 41, *112*, 215, *226*
Nomura, T., 215, *227*
Norman, J. R., 272, *300*, 354, *366*
Norris, E. R., 370, *384*
Nyman, K. J., 230, *267*

O

Öktay, M., 449, *499*
Öztan, N., 443, *499*
Ogneff, J., 324, 330, *343*
Ohshima, H., 355, 356, 358, *366*
Okada, Y., 286, 287, *300*
Okada, Y. K., 351, *366*
O'Leary, J. O., 3, 50, *108*
Olmsted, J. M. D., 198, *208*
Osborn, C. M., 132, 134, 135, *152*, *153*
Osorio, B., 346, *366*
Outram, D. N., 282, *300*
Overton, E., 336, *343*

P

Palay, S. L., 42, *114*, 196, *208*
Papez, J. W., 288, *300*
Parker, G. H., 6, 99, 100, 101, 104, *112*, *113*, 158, 174, 176, 177, 179, *185*, 196, 198, *208*, 371, *384*, 388, *401*
Paton, S., 102, *113*
Patrick, R., 420, *428*
Pavan, C., 467, *499*, *500*
Pavlov, J. P., 213, *226*, *227*
Payne, G. H., 197, *209*
Pearcy, J. F., 51, 52, *113*
Pearse, A. S., 313, *321*
Pecini, M., 475, *495*
Pentelow, F. T. K., 423, *429*
Perdeck, A. C., 254, 255, *268*
Pesson, M., 370, *384*
Peters, H., 230, *267*, 280, *300*
Petravicz, J. J., 295, *300*
Petravicz, W. P., 294, *301*
Pflüger, E., 57, *113*
Pflugfelder, O., 289, *301*
Philipsen, H., 314, *321*
Philippson, M., 236, *267*
Pickford, G. E., 283, 284, *301*, *303*, 479, *500*
Pincher, C., *152*
Piper, H., 179, *185*
Pipping, M., 190, 191, *208*
Plattner, W., 308, 313, *321*
Podkopaew, N. A., 217, *227*
Poggendorf, D., 179, 180, *185*
Polimanti, O., 6, 7, 17, 18, 36, 74, 78, *113*
Polonovski, M., 370, *384*
Polyak, S. L., 147, *152*
Pouchet, G., 97, *113*
Powers, E. B., 309, 311, *321*, 411, 412, 417, *430*

Prechtl, H. F. R., 260, *267*
Priestley, J. G., 310, *319*
Proudfoot, D. A., 315, *320*
Pumphrey, R. J., 147, *152,* 315, *321*

R

Rabl-Rückhard, H., 288, *301*
Rauther, M., 315, 316, *321,* 356, *366*
Rasquin, P., 14, *106,* 129, *152,* 275, 297, *301,* 467, *494, 500*
Ray, D. L., 356, *366*
Razran, G. H. S., 213, *227*
Redeke, H. C., 418, *430*
Redlich, A., 287, *298*
Reeves, C. D., 141, *152,* 294, *301*
Regnart, H. C., 339, *343*
Regnier, H., 287, *301*
Reighard, J. R., 130, *152,* 278, 279, 282, 294, *301*
Reinhardt, F., 181, *185, 227*
Reisinger, L., 6, 17, 38, 39, *113*
Remmler, W., 330, *343*
Remotti, E., 314, *321*
Renzi, P., 6, 36, *113*
Ripplinger, J., 93, 94, 95, 96, 97, 104, *110, 111, 113*
Rivas, L. R., 15, *113*
Rizzo, C., 18, *113*
Rizzolo, A., 7, 17, 37, 49, 50, *114*
Roberts, T. D. M., 174, 175, 181, 182, *184,* 315, *320*
Robertson, O. H., 400, *401*
Robinson, E. J., 274, 275, 291, *301*
Romanes, G. H., 173, *185*
Romieu, M., 15, *114*
Root, R. W., 309, 310, *319, 321*
Rosen, D. E., 291, 292, 293, *301,* 473, *497*
Rosen, D. G., 383, *384*
Rosenblith, W. A., 337, *341*
Rosenbloom, L., 275, *301,* 467, *500*
Rosenblueth, A., 101, *113*
Rosenthal, H. L., 293, *301*, 448, 469, *500*
Rosenthal, R. S., 469, *500*
Rothenberg, M. A., 334, 336, *343*
Roule, L., 279, 281, 282, *301*
Rowan, W., 274, *301*
Rowley, J. B., 133, *152*
Royce, W. F., 275, *301*
Rubin, M. A., 163, *185*
Rugh, R., 274, 275, 291, *301*
Russell, E. S., *224, 227*
Russell, W. M. S., 245, *266*

S

Safford, V., 310, 313, *319, 321*
Sagemehl, M., 314, *321*
Sanborn, N. H., 426, *430*
Sand, A., 56, 57, 71, 73, 100, *109, 114, 152,* 160, 161, 162, 166, 167, 171, 172, 173, *184, 185,* 236, *266*
Sanders, F. K., 34, *114,* 195, *208,* 215, *227*
Saunders, R. S., 311, *321*
Sawyer, C. H., 103, *114*
Sawyer, W., 284, *303*
Schaefer, J. G., 98, *114*
Scharrer, B., 15, *114*
Scharrer, E., 8, 14, 15, 20, 42, *114,* 196, *208*
Schedl, H. P., 275, *299*
Scherrer, J., 33, *107,* 143, *150*
Schlagel, S. R., 147, *152*
Schlosberg, H., 293, 295, *301*
Schlumberger, H. G., 478, *500*
Schmidt, E., 286, *299*
Schmidt, J., 433, 470, *500*
Schmidt, P. J., 355, *366*
Schneider, H., 176, 180, *185*
Schneirla, T. C., 273, 276, 280, 289, *301*
Schoen, L., 30, 104, *114, 118,* 174, *185*
Schönherr, J., 288, 289, 290, *301*
Scholander, P. F., 310, 311, 317, *321*
Schreiber, B., 312, *321*
Schreiner, T., *227*
Schriever, H., 160, *185*
Schroeder, W. C., 338, 339, *341*
Schultz, L. P., 132, *152,* 472, *500*
Schutz, F., 201, *208*
Schwier, H., 445, 448, *500*
Sears, R. R., 215, 216, *227*
Segaar, J., 304, *304*
Seitz, A., 230, 254, 256, *268,* 278, 279, *302*
Senders, L. V., 133, *152*
Sevenster, P., 253, 260, 263, 264, *268*
Sgonina, K., *227*
Shaffer, E. L., 330, *343*
Sheldon, R. E., 6, *114,* 196, 198, *209*
Shelford, V. E., 412, *430*

Shepard, M. P., 414, *430*
Sherrington, C. S., 236, 238, *268*
Shuzo, S., 370, *384*
Simmons, R. W., 370, *384*
Skinner, B. F., 211, *227*
Sklarofsky, B., 303, *304*
Skowron, S., 358, 360, *366*
Slastenenko, E. P., 472, *500*
Smith, C. L., 472, *494*
Smith, G. E., 123, *152*
Smith, G. M., 455, *497*
Smith, M. A., 448, *496*
Smith, O. R., 416, *430*
Smith, P. E., 284, *302*
Smith, S. W., 42, *114*, 196, *208*
Snider, R. S., 41, *114*
Snieszko, S. F., 477, *500*
Sobotka, H., 309, *321*
Sørenson, W., 315, *321*, *322*
Southgate, B. A., 418, 420, *430*
Spaeth, R. A., 99, *114*
Sperry, R. W., 28, 29, 30, 31, 32, 35, *114*, *115*, 129, 130, *152*, *153*
Springer, M. G., 7, 13, 17, *115*
Spurway, H., 446, *500*
Stahl, A., 15, *114*
Stampehl, H., 282, *302*
Stark, J., 323, *343*
Steche, O., 347, 352, *366*
Stefanelli, A., 50, *115*
Steiner, J., 3, 4, 6, 13, 17, 36, 44, 72, 74, *115*, 196, *209*
Steinhausen, W., 170, *185*
Steinmann, P., 160, *185*, 418, *430*
Stensiö, E. A., 158, 168, *186*
Stern, E. M., 132, *152*
Stetter, H., 160, 174, 176, 177, 180, 181, *186*, *228*
Steven, D. M., 382, 383, *384*
Stipetic, E., 176, 180, *186*
Stolk, A., 293, *302*
Strieck, F., 6, *115*, 197, *209*, 221, *227*
Strittmatter, C. F., 309, *318*, *322*
Strong, E. R., 420, *428*
Sugi, M., 215, *227*
Sullivan, C. M., *227*
Sumner, F. B., 371, 375, 382, *385*, 407, *430*
Summerson, W. H., 455, *495*
Surber, E. W., 420, *428*
Svärdson, G., 277, 287, *302*, 470, *500*
Szabo, T., 337, *342*

T

Taft, A. C., 294, *300*
Takase, M., 347, *366*
Taliev, D. N., 478, *500*
Tåning, Å. V., 468, *500*
Tansley, K., 136, 138, 139, 149, *150*
Tarzwell, C. M., 427, *428*
Tauc, L., 334, *342*
Tavolga, W. N., 230, *268*, 279, 280, 281, 282, 283, 284, 285, 287, 292, 295, *302*, 440, 472, 490, *500*
Taylor, H. F., 307, *322*
Teichmann, H., 192, *209*
ten Cate, B., 19, *116*
ten Cate-Kazejewa, B., 67, 71, *116*
ten Cate, J., 3, 6, 18, 19, 37, 46, 47, 48, 52, 53, 54, 55, 65, 66, 67, 68, 71, 72, 73, 74, 85, 104, *115*, *116*, 236, 237, *268*, 288, 290, *302*
Teng, C. T., 311, 317, *321*
ter Pelkwijk, J. J., 230, 253, 261, *268*, 276, 283, *302*
Tester, A. L., 204, *209*
Thibault, C., 135, *153*
Thines, G., 275, *302*
Thompson, D. H., 470, 471, *500*
Thompson, J. A., 433, *495*
Thorpe, W. H., 213, *227*, 234, 245, *268*
Tilney, F., 36, 40, *116*
Tinbergen, N., 230, 232, 233, 245, 247, 248, 253, 254, 255, 261, 263, 264, 265, *267*, *268*, 275, 276, 280, 283, 286, 289, *302*
Tolochinov, I. E., 213, *227*
Tomita, G., 103, *116*
Torrey, T. W., 42, *116*
Toryu, Y., 313, *322*
Tower, R .W., 282, *302*, 315, 316, *322*
Townsend, L. D., 412, 414, *430*
Toyama, K., 433, *500*
Tracy, H. C., 103, *116*
Traube-Mengarini, M., 17, *116*
Trinkaus, J. P., 469, 478, *495*, *496*
Trudel, P. J., 197, *209*, 221, *227*
Tschermak, A., 332, 335, 336, *341*
Tuge, H., 16, 35, 39, 64, 103, *116*
Turner, W. J., 303, *304*

Twitmyer, E. B., 213, *227*

U

Ufland, I., 216, *227*
Ulrich, H., 173, *186*
Umbreit, W. W., 303, *304*
Umrath, K., 49, 100, *116*, 445, *500*

V

van de Kamer, J. C., 12, 15, *116*
van Dam, L., 310, 311, *321*
van den Berghe, L., 191, *209*
Van der Drift, J., 261, *268*
Van der Pyl, M., 360, *364*
van der Sprenkel, H. B., 339, *343*
van der Zee, H., 427, *428*
van Goor, H., 309, *322*
van Herk, A. W. H., 47, 49, *116*
van Heusen, A. P., 177, *185*
Van Horn, W. M., 420, *428*
van Iersel, J. J. A., 230, 232, 234, 235, 242, 243, 244, 246, 250, 263, 265, *268*, 282, *302*
van Rijnberk, G. (van Rynberk, G.), 47, 49, 54, *116*, *117*, *401*
van Rijssel, T. G., 103, *117*
Van Roon, J. M., 261, *268*
van Rynberk, G., *see* van Rijnberk, G.
van Weel, P. B., 204, *209*
Vanden-Eeckhoudt, J. P., 274, *302*
Vanderplank, F. L., *228*
Varé, P., 49, *107*
Vasilenko, T. D., 314, *322*
Vassilenko, P. D., 314, *320*
Vendrik, A. J. H., 171, *183*
Verhagen, T. G., 12, 15, *116*
Verheijen, F. J., 180, *183*, *225*
Verne, J., *401*
Verrier, M. L., 131, 133, 140, 148, *150*, *151*, *153*
Versteegh, C., 173, *182*
Verwey, J., 262, *268*
Vialli, M., 99, *117*
Vilter, V., 100, *117*, 123, 133, 135, *153*
Vivien, J., 284, *302*
Vivier, P., 421, *430*
von Bouteville, K. F., 177, 181, *186*, *228*
von Buddenbrock, W., 3, 30, 54, *117*, 122, 123, 131, 149, *153*
von Frisch, K., 13, 46, 97, 98, 99, *117*, 140, 141, *153*, 160, 169, 174, 176, 177, 178, 179, 181, *186*, 189, 200, *209*, *228*, 253, *268*, 314, *322*
von Gelei, G., 99, *117*
von Hess, C., 140, *153*
von Holst, E., 30, 57, 59, 60, 61, 62, 63, 64, 65, 67, 68, 69, 70, 71, 74, 76, 77, 78, 79, 80, 81, 85, 86, 87, 88, 89, 90, 104, *108*, *117*, *118*, 174, 178, *185*, *186*, 232, 236, 237, 238, 239, 260, *266*, *268*, *269*
von Ihering, R., 284, *302*
von Ledebur, J., 92, *118*, 308, 310, *322*
Voorhoeve, J. J., 40, *118*
Vulpian, A., 6, 17, 36, 39, 43, 44, 57, *118*

W

Wagner, H. G., 143, *151*
Wagner, R. P., 432, *500*
Wakeley, C. P. G., *151*
Walaszek, E. J., 303, *304*
Walcher, H., 100, *116*
Wald, G., 123, 135, 136, 137, 138, *151*, *153*
Walker, K. F., 407, 409, *429*
Walker, T. J., 125, *153*, 202, *209*, 221, *228*
Wallen, I. E., 416, *430*
Walls, G. L., 123, 124, 125, 126, 131, 135, 139, 140, 141, 144, 145, 146, 149, *153*, 279 *302*
Walsh, J., 323, *343*
Walters, V., 311, 317, *321*
Waring, H., 100, *118*
Warner, L. H., 141, 149, *153*
Wasteneys, H., 407, *429*
Waterbolk, H. Tj., 230, 234, 249, 258, 259, 264, *265*, 282, 293, *296*
Waterman, T. H., 354, 355, *366*
Watson, J. B., 213, *228*
Weber, A., 3, *118*
Weber, H., 74, *118*
Weill, R., 348, *366*
Weiss, P., 32, 51, 79, 85, *111*, *118*, 238, 251, *268*, 273, *303*
Welch, J. H., 132, 134, 135, *153*
Werner, C. F., 174, *186*
Westerfield, F., 176, 180, *186*

Westfall, B. A., 418, 422, *430*
Westoll, T. S., 307, *322*
White, E. G., 327, 329, *343*
White, E. I., 158, *186*
White, G. M., *153*
White, M. J. D., 433, *500*
Whitear, M., 97, *118*
Whiteley, G. P., 197, *209*
Whiting, H. P., 101, 102, 103, *109*, *118*
Whitman, C. O., 231, *269*
Wickbom, T., 443, *500*
Wiebe, A. H., 412, 415, *430*
Wilhelmi, A., 284, *303*
Williamson, H., 323, *343*
Willmer, E. N., 310, *322*
Wilson, D. P., 262, *269*, 338, *343*
Winge, O., 433, 435, 437, 438, 448, *501*
Winn, H. E., 276, 294, 295, *297*, *303*, 472, *494*
Winterstein, H., 78, *118*
Wintrebert, P., 102, *118*
Wisby, W. J., 202, 203, 204, 205, 206, *207*, *208*, *209*, 221, *226*, *228*
Wohlfahrt, T. A., 180, 181, *186*, *228*
Woker, H., 421, 422, 423, 427, *430*
Woldring, S., 43, 46, *118*
Wolf, E., 139, 140, 149, *151*, 466, *494*
Wolf, L. E., 446, 476, *501*
Wrede, W. L., 199, *209*, *228*
Wuhrmann,K., 421, 422, 423, 427, *430*
Wulfson, S. H., 212, *228*
Wunder, W., 123, 132, 135, 140, *153*, 158, *186*, 191, 197, *209*, *228*, 230, *269*, 314, *322*
Wykes, U., 14, *119*
Wyman, L. C., 97, *119*

Y

Yagi, K., 370, *385*
Yamashita, H., 286, 287, *300*
Yasaki, Y., 351, *366*
Yoshino, S., 160, 162, *183*, *184*
Young, J. Z., 14, 91, 92, 97, 100, 101, *119*, 125, 128, 129, 131, 148, *154*, 275, *303*, 312, *322*
Young, R., 475, *498*
Young, R. T., 382, *385*
Youngstrom, K. A., 101, *119*

Z

Zehender, F., 421, 422, *430*
Zenneck, J., 177, 179, *186*
Zerrahn-Wolf, G., 466, *494*
Zunini, G., *228*

# SUBJECT INDEX

A

*Abramis brama* (bream), 134, 276
Abyssal fish, see Bathypelagic fish
*Acanthias vulgaris,* see *Squalus acanthias*
*Acerina cernua* (pope, ruffe), 159, 177, 190
Acetylcholine (ACh), 94–97, 102, 104, 312, 334–336, 394
Acids, effect on fishes, 45, 422, 423
*Acipenser* (sturgeon), 195
Acoustico-lateralis system, see also Balance, Sound, 43, 155–182
  ampullae of Lorenzini, 156, 164–168
  ear, 29, 31, 37, 42, 168–182, 161
  lateral line, 20, 42, 50, 58, 156–164, 168, 339
  nerves, fibers and centers, 16, 36, 59, 157, 159, 161–164, 171, 173, 181, 182
  vesicles of Savi, 156
  Weberian ossicles, 179, 181, 312, 314, 317
*Acropoma japonicum,* 353, 362
Acropomatidae, 349, 353
Adrenaline, 99, 100, 312, 360, 394, 395
*Aequidens,* 240, 249
  *A. latifrons* (blue acara), 284
  *A. maroni,* 264
Agnatha, see also Cyclostomata, 122, 123
*Alburnus alburnus (A. lucidus)* (bleak), 57, 74, 137, 177
*Alosa alosa* (shad), 281
*Amblypharyngodon,* 275
*Ameiurus* (bullhead, catfish), 6, 43, 50, 134, 135, 143, 161, 163, 181, 193–196, 198, 220, 222, 389, 392–397, 399, 400
  *A. nebulosus,* 14, 27, 32, 33, 100, 101, 132, 159, 177, 180, 200, 230, 279, 407–409
*Amia* (bowfin), 139
Amino acids and pigments, 369–371
Ammocoete larva, see Cyclostomata
*Ammodytes (Hyperoplus) lanceolatus,* 381
  *A. tobianus,* 381
Ammonia, toxicity to fishes, 406, 422
*Amphipnous fossorius,* 147
*Amphiprion* (coral fish), 262
Ampullae of Lorenzini, see Acoustico-lateralis system
Anabantidae, 176, 180, 272, 380
*Anabas scandens (A. testudineus),* 176
*Anableps anableps* (four-eyed fish, top-minnow), 145, 146
*Anatolichthys,* 443
*Ancylopsetta* (flounder), 134
*Angelichthys isabelita* (angel fish), 479
Anglers, see Ceratioidea, Lophiidae, *Lophius*
*Anguilla* (eel), 125, 134, 137, 148, 192, 193, 222, 317, 389, 397
  *A. anguilla* (European eel), 57, 59–64, 66–70, 74–76, 78, 80, 176, 180, 190, 306, 307, 315, 369, 376
  *A. japonica,* 162
  *A. rostrata* (American eel), 315
Anomalopidae, 350, 352, 354
*Anoptichthys hubbsi* (cave fish), 467
  *A. jordani,* see *Astyanax mexicanus*
*Aphanius,* 443
*Aphia minuta,* 380
*Aplocheilus latipes,* see *Oryzias latipes*
*Apogon marginatus,* 354
*Argentina,* 317
*Argyropelecus hemigymnus,* 356
  *A. olfersi,* 359
*Astatotilapia strigigena,* see *Haplochromis multicolor*
*Astrape japonica* (electric ray), 331
*Astronesthes,* 346, 356
*Astronotus ocellatus,* 264
*Astroscopus guttatus* (American stargazer), 324, 325, 327, 329–331, 338
*Astyanax mexicanus (Anoptichthys jordani),* 466
Atropine, 97, 312

Autonomic nervous system (sympathetic system), 12, 15, 21, 49, 50, 90–101, 307, 312, 362, 363, 395

B

Balance (equilibrium, posture), 6–8, 16, 17, 19, 22, 23, 29, 37–39, 41, 43, 104, 169–175, 178, 260
Balistidae (trigger fish), 315
*Barbus* (see also *Caecobarbus*), 275
  *B. nigrofasciatus,* 377
*Bathygobius soporator* (goby), 35, 130, 279, 281, 282, 284, 285, 287, 295
*Bathylagus,* 144
Bathypelagic fishes, 126, 144, 147, 317, 348
Batrachoididae, 348, 349, 360
Behavior, 211–304 (see also Reflexes)
  appetitive, 236, 248–252, 273
  conditioned, 8, 10, 34, 35, 41, 140, 141, 197–200, 202–206, 211–224
  displacement activity, 247, 248, 263, 273
  effect of drugs on, 303, 304
  instincts, 230, 231, 233, 234, 239, 240–252, 258–260, 263–265, 272, 273
  learned, 34, 35, 40, 41, 230, 231, 256, 273
  releasing mechanisms, 252–265
  vacuum activity, 235
*Belone,* 94, 95
*Benthobatis moresbyi* (blind torpedo), 339, 356
*Beryx decadactylus,* 379
*Betta splendens* (Siamese fighting fish), 176, 230, 254, 260, 279, 283, 285–287, 289, 303, 380, 445, 448
Bilichromes, 367, 368
Bitterling, see *Rhodeus amarus*
Blennies, Blennioids, see *Ammodytes, Blennius, Pholis, Zoarces*
*Blennius,* 124, 133, 222
  *B. gattorugine,* 221, 222, 369
  *B. pavo,* 279, 282
  *B. pholis,* 220, 221, 223
Blood, dissociation curves of oxyhemoglobin, 309, 310
  supply to eye, 125, 126
    to swimbladder, 307, 309–311, 317
*Boleophthalmus* (mudskipper), 145
*Boleosoma nigrum* (Johnny darter), 294, 471, 472
*Bothus (Rhomboidichthys),* 47
  *B. maximus (Scophthalmus maximus) (Psetta maxima)* 190, 378
  *B. rhombus (Scophthalmus rhombus),* 379
Brain, see also Diencephalon, Medulla, Mesencephalon, Metencephalon, Telencephalon
  anatomy, 3–5, 12, 13, 16, 35, 36, 42, 43
  cerebral cortex (of mammals), 4, 16, 35, 40, 214, 215, 288, 289
  cerebrospinal fluid, 103
  electric lobe, 327, 337
Bullhead, see *Ameiurus, Cottus gobio*

C

*Caecobarbus* (cave fish), 467
Caffeine, theine, 45, 50
Californian toadfish, see *Porichthys notatus*
*Callionymus lyra* (dragonet), 133, 308, 381
Carangidae (jacks, yellowtails), 315, 379
*Caranx trachurus,* 379
*Carassius auratus* (goldfish), 8–10, 18, 34, 39–41, 43, 45, 51, 52, 57–59, 61, 64, 74, 76–78, 133, 174, 177, 195, 238, 303, 308, 313, 370, 374, 377, 407–409, 412, 417, 418, 473, 477, 478
  *C. carassius,* 50, 51, 57, 177
  *C. gibelio* (carp), 103
Carbon dioxide, effect on fanning of *Gasterosteus,* 234, 243, 254
  in natural waters, 411–413, 419, 421, 425, 426
  in swimbladder, 305, 309–311
*Carcharinus commersonii* (blue shark), 368, 369
Carotenoids, 367, 371–383
Carp, see Cyprinidae, *Carassius gibelio, Cyprinus carpio*
Castration, see Gonadectomy
Catfish, see Siluridae, *Ameiurus, Parasilurus, Pimelodella*
  electric, see *Malapterurus*
Cave fishes, see *Astyanax, Caecobarbus,*

*Typhlobagrus, Typhlogobius,* 147, 303, 466, 467
Centrarchidae (sunfishes), 230, 274, 278, 415
*Centrolabrus exoletus* (wrasse), 379
*Centronotus gunnellus,* 222, 223
*Centropristes* (sea bass), 446
  *C. striatus.* 379
*Cephalaspis,* 122
Ceratioidea (anglers), 348, 349, 354, 355, 364, 371
Cerebellum, see Metencephalon
Cerebral cortex, see Brain
*Cetorhinus* (basking shark), 144
Char, see *Salvelinus*
Characinidae, 177, 263, 275, 467
*Chauliodus sloanii,* 346, 348, 358, 360
*Chimaera,* 149
*Chirolophis galerita,* 222, 223
Chlorine, 426, 427
Chloroform, 45
Choline, 99
Choline esterase, see Enzymes
Chondrichthyes, see Elasmobranchii
Chromatophores, 383, 387–400, 447–464, 475, 478, 492
  genes affecting, 447–454, 458–465, 474, 478, 492
  iridophores, 388, 390, 391
  nervous control of, 13–15, 21, 46–50, 97–101, 104, 393–395, 397
Chromosomes and sex, 433–444
Chub, see *Leucosomus, Squalius*
*Cichlasoma,* 240, 249
  *C. meeki,* 256, 264
  *C. nigrofasciatum,* 251, 262
Cichlidae, 230, 235, 240, 245, 249, 253, 255–257, 262, 263, 272, 278, 283, 291, 294, 295
*Ciliata mustela,* see *Gaidropsarus mustelus*
*Clupea harengus* (herring), 376
  *C. pallasii* (Pacific herring), 282, 412
Clupeidae (herrings), 123, 131, 181, 272, 317
Cobitidae (loaches), 314
*Cobitis fossilis,* see *Misgurnus fossilis*
  *C. taenia* (spiny loach), 491
Cocaine, 7, 38, 49, 55
Cod, see *Gadus callarias*
*Coelorhynchus coelorhynchus,* 347, 350
*Colisa fasciata,* 380
  *C. lalia,* 176, 380
Color, see Chromatophores, Eye, names of classes of pigments
*Conger conger* (conger eel), 94, 95, 142, 308, 369
*Copeina guttata,* 377
*Coregonus* (whitefish), 276, 295
  *C. albula,* 376
*Corvina nigra,* 159, 177, 180
Cottidae (sculpins), 176, 317
*Cottus,* 222
  *C. bubalis,* 223, 308, 380
  *C. gobio* (bullhead), 176, 230
  *C. scorpius,* 176, 190, 223
*Crenilabrus* (wrasse), 6, 64, 65, 95, 222, 389
  *C. griseus,* 176
  *C. melops,* 176, 223, 308, 379
  *C. pavo,* 176
*Ctenolabrus suillus,* 380, 396
Curare, 7, 19, 38, 333, 334
Cyanide, 417, 419, 423, 424
*Cyclopterus lumpus* (lumpsucker), 223, 380
Cyclostomata (hagfishes, lampreys), 3, 4, 14, 35, 72, 90, 91, 93, 94, 102, 123, 168, 196, 374, 396
*Cyclothone acclinidens,* 348, 356
*Cymatogaster aggregata* (yellow-banded, viviparous surf perch), 176, 379, 382
*Cynoscion regalis* (weakfish), 174, 282
Cyprinidae, 126, 133, 201, 263, 275
*Cyprinodon variegatus,* 479
Cyprinodontidae, 176, 230, 286, 377, 443, 446
*Cyprinus carpio* (carp), 17, 33, 36, 39, 43, 46, 57, 64, 97, 131, 135, 137, 139, 148, 193, 195, 197, 369, 370, 449, 470, 478

D

Darters, see Etheostomidae, *Boleosoma, Hadropterus*
*Dasyatis pastinaca (Trygon vulgaris, Raia pastinaca),* 7, 19, 37, 49
Detergents, 427
Development, of acoustico-lateralis sys-

tem, 155, 168
  of behavior, 252, 256
  of electric organs, 328, 329
  of eye, 123
  effect of genes on, 447–449, 458
    of pH on, 411
    of silt on, 416
    of temperature on, 468–471
  of olfactory sacs, 189, 190
  of melanomas, 448, 452, 455–458, 463, 464
  of nervous system, 101–103
  of swimbladder, 305
*Diaphus,* 347
Diencephalon (tweenbrain), 4, 5, 12–16
  infundibulum, 12
  lobi inferiores, 4, 5, 12, 28
  pineal organ (epiphysis), 12, 14, 275, 288, 396, 397
  thalamus, 4, 12, 13, 16, 122, 275, 288, 290, 393
Dipnoi (lungfishes), 3, 4, 189, 307
Dogfish, see *Etmopterus, Galeus, Mustelus, Scyliorhinus, Squalus*
*Dolopichthys,* 354
Drugs (and see under individual drugs)
  autonomic, 92, 93, 97, 394
Drumfish, see Sciaenidae

E

Ear, see Acoustico-lateralis system
*Echiostoma cyenobarba,* 349, 359, 363
Eel, see *Anguilla,* Saccopharyngidae, *Rhynchocymba*
  American, see *Anguilla rostrata*
  conger, see *Conger conger*
  electric, see *Electrophorus electricus*
  European, see *Anguilla anguilla*
  moray, see *Muraena*
Eel-pout, see *Lota, Zoarces*
Eggs, pigments in, 368, 372, 373, 383
*Eigenmannia virescens,* 332
Elasmobranchii, 3–7, 16, 17, 35–38, 47, 54–57, 72–74, 91–94, 97, 102, 103, 123, 125–128, 131, 136, 144, 147, 148, 156, 158, 164–166, 168, 171, 173, 182, 188, 197, 291, 292, 323, 324, 334, 349, ,355, 356, 363, 368, 374, 392, 397, 453
Electric organs, 50, 51, 323–340
  discharge of, 329–336
  evolution of, 261, 339
  innervation of, 327, 328, 334, 336–338
  origin and development, 328, 329
*Electrophorus electricus (Gymnotus electricus)* (electric eel), 51, 177, 323–340
Electroplates (electroplaxes), 324–337
*Eleginus navaga,* 377
*Enchelyopus cimbrius,* see *Gaidropsarus cimbrius*
Endocrines, see under names of organs
*Entelurus aequorus,* see *Nerophis aequorus*
Enzymes, 492
  carbonic anhydrase, 309
  choline esterase, 312, 334, 394
  dopase, 450
  tyrosinase, 455
*Epigonus,* 144
*Epinephelus mystacinus* (black grouper), 310
Epiphysis, see Diencephalon, pineal organ
*Equula,* see *Leiognathus*
Ergot, 99, 303
Ergotamine, 99, 100
Eserine, 97, 103
*Esox lucius* (pike), 39, 43, 95, 137, 139, 149, 158, 164, 170, 172, 173, 190, 192, 193, 253, 261, 262, 274, 377
*Etheostoma coeruleum* (rainbow darter), 294
Etheostomidae (darters), 283, 294, 295, 317
*Etmopterus lucifer* (spiny dogfish), 355
  *E. spinax (Spinax niger),* 347, 355, 356
*Eupomotis* (sunfish), 162
Eye, 121–150, see also Light
  anatomy, 123–130, 148, 149
  color vision, 138, 140, 141, 220
  functioning of, 123, 128, 130–150
  genetics of eye characters, 464, 465, 492
  loss of, 147, 466, 467
  optic tract (nerve) (cranial nerve II), 4, 5, 13, 16, 21, 25, 28, 30, 32, 128–130, 141, 143
  sight in relation to the nervous system, 6, 8, 10, 13, 16, 17, 20–32, 34,

35, 39, 41, 79, 104 (see also Mesencephalon)
  pigments in, 126, 127, 134–138, 147, 149, 377, 379, 380, 382

F

Feeding, and electric organ, 338
  nervous basis of, 6, 8, 11, 17, 24, 26
Fighting fish, Siamese, see *Betta splendens*
Fins, see Movements
Flatfish, 125, 148, and see *Bothus, Platichthys, Pleuronectes, Solea*
Flavines, 367–369
Flounder, see *Ancylopsetta, Limanda, Paralichthys, Platichthys, Pseudopleuronectes*
*Fundulus* (killifish, topminnow), 6, 99, 134, 137, 276, 295, 382, 389, 390, 392, 396, 397, 399, 469
  *F. heteroclitus,* 97, 100, 103, 176, 283, 284, 308, 398, 468, 479
  *F. notatus,* 294
  *F. parvipinnis,* 375, 377, 409

G

Gadidae, 176, 272, 350
*Gadus,* 222
  *G. aeglefinus (Melanogrammus)* (haddock), 176, 377
  *G. callarias (G. morrhua)* (cod) 92, 176, 195, 223, 305, 377, 378
  *G. luscus,* 308
  *G. merlangus* (whiting), 223, 378
  *G. minutus (Trisopterus minutus),* 378
  *G. pollachius (Pollachius pollachius)* (pollack), 283, 378
  *G. virens (Pollachius virens)* (coalfish), 223, 378
*Gaidropsarus cimbrius (Enchelyopus cimbrius),* 378
  *G. mustelus (Ciliata mustela),* 378
*Galeus canis* (smooth dogfish), 7, 17, 37, 49
*Gambusia affinus* (mosquito fish), 99, 407, 445, 452, 472
Gasterosteidae (sticklebacks), 261, 272
*Gasterosteus aculeatus* (three-spined stickleback), 8, 16, 36, 190, 192, 222, 230, 232–236, 238, 242–250, 252–254, 263, 265, 274–276, 279–283, 285, 286, 288–290, 295, 304, 381, 470, 471
*Gazza,* 353
Genetics, 432–493
Geotaxis, 260
*Gillichythys mirabilis* (long-jawed goby), 380, 382
Gills, and poisons, 416–419, 425, 426
  tumors of, 479, 483, 484
*Girella nigricans* (greenfish, opaleye), 380, 382, 406–409
*Gnathonemus,* 176, 180
Gobies, see Gobiidae, *Bathygobius, Gillichthys, Gobius, Typhlogobius*
Gobiidae (gobies), 147, 176, 230, 282, 283, 287, 291, 380
*Gobio fluviatilis* (gudgeon), 10, 192
*Gobius* (goby), 222, 392, 396
  *G. capito* (giant goby), 64
  *G. flavescens,* 222, 223
  *G. jozo,* 174
  *G. lota,* 45
  *G. niger,* 176, 180, 190, 380
  *G. paganellus,* 176, 284
Goldfish, see *Carassius auratus*
  marine, see *Hypsypops*
Gonads, 283–285, 393, 439, 441–444, 446, 458, 467
  androgens, including testosterone, 284, 286, 287, 304, 435
  estrogens, 286–288
  gonadectomy (castration) and breeding, 283–286, 304, 393
  pigments in, 372, 373, 376–383
  progesterone, 286, 287
Gonostomatidae, 349
Gourami, see Anabantidae, *Macropodus, Trichopsis*
Guppy, see *Lebistes reticulatus*
Gustatory sense, see Taste
Gut, nervous control of, 91, 92
*Gymnarchus niloticus,* 323–325, 327, 329–332, 339
*Gymnocymbus ternetzii,* 174
*Gymnorhamphichthys postumus,* 332
Gymnotidae, 177, 324, 325, 328, 329, 331, 332, 336, 339
*Gymnotus,* 324
  *G. carapo,* 330–332

- *G. electricus*, see *Electrophorus electricus*

H

- *Hadropterus maculatus* (black-sided darter), 294
- Haemulidae, 315
- *Haplochromis multicolor (Astatotilapia strigigena)*, 254, 256, 279, 280
- Hearing, see Acoustico-lateralis system, Sound
- Heart, nervous control of, 45, 50, 90, 91, 93–97
- *Hemichromis bimaculatus* (jewel fish), 240, 249, 253, 256, 277–279, 281, 285, 287–289
- *Hemigrammus caudovittatus*, 177
- Herring, see *Clupea*
- *Heterandria*, 201
- *Hippocampus* (seahorse), 40, 74, 238, 282
- *Hippoglossus hippoglossus*, 379
- *Histrio histrio* (sargassum fish), 278
- *Holocentrus*, 6, 391
- Holocephali, 91
- Hormones, 400, 449, see also under names of endocrine glands
- *Hyborhynchus notatus* (bluntnose minnow), 198, 202–205, 221, 407
- Hybrids, 440–445, 450, 451, 453–458, 462–466, 472, 473, 475, 478, 486–490
- *Hydrolagus*, 91
- *Hyperoplus lanceolatus*, see *Ammodytes lanceolatus*
- *Hyphessobrycon flammeus*, 177
- Hypophysectomy, see Pituitary
- Hypothalamus, see Diencephalon, Thalamus
- *Hypsypops rubicunda* (Garibaldi, marine goldfish), 375, 379

I

- *Idus melanotus (Leuciscus idus)*, 177
- Indoles, 367, see also Melanins
- Iris, 91, 97

J

- Jewel fish, see *Hemichromis bimaculatus*
- *Julis giofredi*, 140

K

- *Kiaeraspis*, 168
- Killifish, see *Fundulus* and Cyprinodontidae
- *Kosswigichthys*, 443
- *Kryptophaneron*, 352

L

- Labridae (wrasses), 136, 176, 379
- *Labrus*, 64, 65
  - *L. bergylta*, 380
  - *L. festivus*, 86–88
  - *L. ossifagus*, 380
- *Lampanyctus leucopsarus*, 356
- *Lampetra* (lamprey), 131, 261, 279, 282, 397
  - *L. fluviatilis* (river lamprey), 14, 102, 376
  - *L. planeri* (brook lamprey), 376, 396
  - *L. wilderi* (brook lamprey), 279
- Lampreys, see Cyclostomata, *Lampetra, Petromyzon*
- *Lampris regius* (opah), 374, 378
- Lateral line, see Acoustico-lateralis system
- Learning, see Behavior
- *Lebistes reticulatus* (guppy), 52, 133, 176, 201, 231, 234, 235, 246, 249, 250, 258, 259, 261, 264, 279, 282, 287, 289, 292–294, 303, 389, 399, 433–435, 437–440, 445, 446, 448, 469, 470, 493
- *Leiognathus (Equula)*, 353, 354, 362
- *Lepadogaster*, 396
- *Lepisosteus (Lepidosteus)*, 125
- *Lepomis* (sunfish), 122, 139, 141, 148, 162, 282, 443
  - *L. cyanellus* (green sunfish), 304, 442
  - *L. gibbosus* (common sunfish), 442
  - *L. macrochirus* (bluegill), 442
- *Leuciscus dobula*, 177
  - *L. erythrophthalmus*, see *Scardinius erythrophthalmus*
  - *L. idus*, see *Idus melanotus*
  - *L. rutilus*, see *Rutilus rutilus*
- *Leucosomus* (chub), 126
- Light, see also Eye
  - effect on gonads, 122, 274, 275
  - production by fishes, 345–364
  - visual stimuli affecting behavior, 220,

253–257, 260, 261, 263, 264, 274–276, 278–281
*Limanda limanda* (dab, flounder), 379
*Limia,* 445
  *L. caudomaculata,* 445
  *L. nigrofasciata,* 445
  *L. vittata,* 445
*Linophryne arborifer,* 355, 360
*Liparis montagui,* 223
Liver, 372, 373, 377, 378, 380–382
Loach, see Cobitidae, *Cobitis, Misgurnus*
Locomotion, nervous control of, 6, 13, 19, 22–32, 36–40, 50–90, 236, 237
Lophiidae (anglers), 125, 262
*Lophius piscatorius* (angler fish), 40, 128, 262, 381
*Lophopsetta,* 395
*Lophotus* (crestfish), 371
*Lota lota* (burbot, eelpout), 132, 192, 195, 374, 378
*Lovettia seali* (Tasmanian whitebait), 371
Lungfish, see Dipnoi

**M**

*Macropodus* (gourami, paradise fish), 103, 282, 374, 448
  *M. chinensis,* 445
  *M. concolor,* 445
  *M. cupanus,* 176
  *M. opercularis,* 176, 180, 380, 469
Macrouridae, 347, 350
*Malacocephalus laevis,* 347, 348, 350
*Malapterurus electricus,* (electric catfish), 323, 324, 326–332, 335, 337–339
*Marcusenius isodori,* 176, 180
*Maurolicus pennantii,* 357, 358
Medaka, see *Oryzias latipes*
Medulla oblongata, 4, 13, 16, 36, 38, 42–46, 50, 55, 59, 64, 71–77, 79, 84, 86, 98, 99
  Mauthner cells, 50
Melanin, 367, 370, 371
*Melanocetus johnsoni* (black sea devil), 371
Melanophores, see Chromatophores
Mesencephalon (mid-brain), 4, 5, 7, 12, 13, 16–35
  tectum, 16–35
  tegmentum, 16, 22, 24
Metencephalon (cerebellum, hindbrain), 4, 5, 13, 16, 18, 35–41, 290, 339
  cerebellar peduncle, 37, 38, 72
*Micropogon undulatus,* 315
*Micropterus dolomieu* (smallmouth bass), 413
Microstomidae, 317
*Microstomus kitt (Pleuronectes kitt),* 379
Migration, 204–206, 224, 249, 250, 276
Minnow, see *Hyborhynchus, Notropis, Phoxinus, Pimephales*
*Misgurnus anguillicaudatus* (mud loach), 491
  *M. (Cobitis) fossilis* (loach), 59, 67, 78, 164, 222
*Mola mola,* see *Orthagoriscus mola*
*Mollienesia* (mollies), 445, 452
  *M. formosa,* 445
  *M. latipinna,* 445
  *M. sphenops,* 445
*Monocentris japonica* (knightfish), 350, 351
Mormyridae (elephant snoutfish), 40, 176, 180, 324, 325, 329, 331, 339
*Mormyrus,* 323, 324, 327, 328
  *M. oxyrhynchus,* 330
*Morone* (white perch), 137
Morphine, 49
Movements, nervous control of,
  "circus" (forced optokinetic), 18, 28–30, 37–39, 43
  of embryo, 101–103
  eye, 7, 9, 10, 22, 23, 42, 43, 169, 170, 172, 174
  fin, 7, 18, 19, 23, 36–38, 43, 49, 54–56, 64, 65, 74, 77–79, 86–90, 237
  locomotory 6, 13, 16–19, 22–24, 36, 37, 58, 66–74, 77–79
  of ovaries, 93
  respiratory, 7, 13, 17, 42–45, 72, 77–79
*Mugil* (mullet), 195
  *M. auratus,* 7, 18, 308
*Muraena helena* (moray eel), 369
Muscles, alarm substance in, 200
  innervation, 46–49, 53
  pigments in, 382, 383
*Mustelus* (dogfish), 95, 128, 148, 196, 198, 395
  *M. canis,* 7, 13, 174, 397

*M. "vulgaris,"* 37, 73
Myctophidae (Scopelidae), 348, 349, 358
Myctophoidea, 348, 356
*Myctophum,* 356, 360
*M. affine,* 360
*M. watasei,* 358
*Myliobatis aquila* (eagle ray), 7, 19, 37, 128, 148
*Myxine glutinosa* (hagfish), 40, 93, 397

N

*Narcine* (electric ray), 324
*N. brasiliensis,* 327, 330, 338
*Nemacheilus barbatula,* 164, 177, 195, 196, 222
*Neoscopelus,* 349
*Nerophis,* 222
*N. aequorus (Entelurus aequorus),* 381
*N. lumbriciformis,* 223
*N. ophiodon,* 190, 381
Nerves, cranial I (olfactory), 16, see also Olfaction
cranial II (optic), see Eye
III (oculimotor), 86, 91, 97, 327
V (trigeminal), 16, 42, 91, 98, 159, 160, 356
VI (abducens), 42, 337
VII (facialis), 40, 42, 44, 46, 157, 159, 160, 165, 195, 196, 356
VIII (acusticus), 16, 36, 37, 42, 43, 50, 72, 73, 171
IX (glossopharyngeus), 42, 44, 46, 72, 73, 157, 195, 196
X (vagus), 36, 42, 44–46, 73, 86, 91–97, 157, 159, 195, 312, 339
chromatic, 97–101, 393, 397
ciliary, 97
gustatory, see Taste
spinal, 46, 48–65, 67, 69, 80, 82–85, 97, 98, 356
visceral (see also Autonomic nervous system), 4, 42, 92
Neurohumors (see also Acetylcholine, Adrenaline), 394, 395, 397, 400
selachine, 395, 397
Neurones, controlling locomotory rhythm, 74, 81
of electric nerves, 327
Neurosecretory cells, 15
Nicotine, 49, 97
Nitrogen, effect on fishes, 415, 416
in swimbladder, 305, 309, 311
*Notemigonus crysoleucas* (golden shiner), 126, 472
*Notropis blennius* (minnow), 141, 407, 410

O

Olfaction, organs of, 3, 4, 6, 188–193
stimuli affecting behavior, 200, 201, 204–207, 253, 255, 281, 282
perceived by organs, 187–188, 193–195, 243, 244, 254, 261, 279, 281, 282
tract, 4, 5, 193–195
bulb, 4, 5, 11, 12
*Oncorhynchus* (Pacific salmon), 197, 370, 374, 407, 409
*O. keta* (chum salmon), 287
*O. kisutch* (coho, silver salmon), 192, 201, 205, 206, 287
*O. nerka* (sockeye salmon), 206, 377, 409
*Ondostomus hyalinus,* 145
*Onos mustela,* 222, 223, 308
*Ophiodon elongatus* (lingcod), 126
*Opisthoproctus soleatus,* 145
*Opsanus tau* (toadfish), 103, 282, 313, 316
Osmoregulation, 409, 410, 411, 419
Ostariophysi, 177, 179–182, 312, 314–317
*Orthagoriscus (Mola) mola,* 381
*Oryzias latipes* (medaka), 64, 139, 274, 286, 291, 377, 433–435, 440, 448, 449, 469, 472, 473
Oxygen, concentration in freshwater, 404–406, 413, 416
dissociation curve of blood, 309, 310
and dopa, 370
effect on behavior, 78, 275, 282
on fluorescyanin, 370
requirements of fish, 405, 406, 408, 412–415, 421, 491
supply to cornea and retina, 124, 126
in swimbladder, 305, 309, 311–313

P

Pacini's Law, 332, 335
*Paralichthys* (flounder), 134, 141, 397, 399
*Parasilurus asotus* (catfish), 393, 394

*Paratrachichthys prosthenius*, 353
Parthenogenesis, 445, 446
*Perca flavescens* (yellow perch), 162, 313, 407
  *P. fluviatilis* (river perch), 6, 17, 38–40, 43, 92, 177, 190–192, 200, 308, 313, 374, 379
Perches, see Embiotocidae, Percidae
  European, see *Perca fluviatilis*
  white, see *Morone*
  yellow, see *Perca flavescens*
Percidae, 126
*Percina caprodes* (logperch), 278
*Periophthalmus koelreuteri*, 145, 148, 149, 176
Peristalsis of gut, 92
*Petromyzon* (lamprey), 72, 94, 172, 173
  *P. marinus* (sea lamprey), 137, 369, 397
pH, effects on fishes, 222, 248, 411–413, 421–426
Phenol, effect on fishes, 417, 427
*Pholis gunnellus* (blenny, gunnel), 381
Phosphocreatine, 336
*Photoblepharon*, 347, 352, 353, 362
Phototaxis, 15, 260, 467
*Phoxinus phoxinus (P. laevis)* (minnow), 6, 8, 10–15, 17, 20, 21, 34, 39, 41, 46, 74, 97–99, 104, 135, 141, 149, 159, 160, 169, 170, 174, 177, 180, 181, 189, 190, 192, 197, 199–201, 204, 217, 221–223, 253, 314, 315, 392, 393, 396
*Phrynorhombus norvegicus*, see *Scophthalmus norvegicus*
*Physiculus japonicus*, 350
Picrotoxin, 50
Pike, see *Esox lucius*
*Pimelodella transitoria* (catfish), 467
*Pimephales* (minnow), 141, 407
  *P. notatus*, 177
  *P. promelas*, 424
Pineal organ (epiphysis), see Diencephalon
Pituitary (hypophysis), 14, 15 ,122, 275, 288, 396, 397
  gonadotropins, 283, 286, 304
  hormones, 283, 284, 391, 392
  hypophysectomy, 283, 284, 391, 392, 397, 400
  intermedin, 392, 393, 397, 398
  oxytocin and vasopressin, 284
*Platichthys (Pleuronectes) flesus* (flounder), 49, 223, 379
Platyfish, see *Xiphophorus maculatus*
*Platypoecilus maculatus*, see *Xiphophorus maculatus*
  *P. variatus*, see *X. variatus*
*Pleuronectes*, 98, 222, 392, 397
  *P. flesus*, see *Platichthys flesus*
  *P. kitt*, see *Microstomus kitt*
  *P. limanda*, see *Limanda limanda*
  *P. platessa* (plaice), 223, 308
Pleuronectidae (flatfish), 40
*Plotozus anguillaris*, 164, 165
Poeciliidae (topminnows), 201, 279, 287, 291–293, 295, 374, 434, 473
*Polistotrema*, 93
*Pollachius pollachius*, see *Gadus pollachius*
  *P. virens*, see *G. virens*
*Polyipnus*, 347, 349
Polypteridae (bichirs), 307
*Porichthys notatus* (Californian toadfish), 347, 348, 360–363
Posture, see Balance
Pressure, effect on fish breeding, 275
  and swimbladder, 307–309, 311–314, 316, 317
*Prionotus carolinus*, 380
*Pristella riddlei*, 263
Proprioceptors, 18, 55, 73, 76, 78, 79, 81, 85, 87
*Psetta maxima*, see *Bothus maximus*
*Pseudopleuronectes americanus* (flounder), 174, 295, 392, 393, 397
*Pteraspis*, 123
Pterins, 367, 369, 370
*Pungitius*, see *Pygosteus*
Purines, 367–369
*Pygosteus (Pungitius) pungitius* (ten-spined stickleback), 190, 253, 260, 263, 295
*Pyrrhulina rachoviana*, 177

R

*Raia (Raja)* (skates, rays), 44, 85, 161, 166, 167, 291, 324, 326–328, 335, 337
  *R. batis*, 328, 376
  *R. clavata*, 47, 48, 104, 162, 171, 174, 175, 181, 315, 329–331, 334, 376

*R. erinacea,* 55
*R. radiata,* 55
*R. undulata,* 37
*Raniceps raninus,* 223, 378
Rays, see *Astrape, Dasyatis, Myliobatis, Narcine, Raia*
Reflexes, 8, 9, 22, 36, 46, 50, 73, 79, 101–103, 331, 336, 338
chain, 66–69
conditioned, see Behavior
locomotory, 64, 67, 68, 76, 78, 81, 85
orienting ("taxes"), 232–234, 239, 252, 260
plurisegmental, 54–65
postural, 22–24, 26–32, 41, 169–172, 174, 178
unisegmental, 53, 54
visceral, 45
"visual grasp," 22–24, 26, 27
*Regalecus glesne* (oarfish), 378
Respiration, nervous control of, 7, 8, 13, 43–46, 50, 72, 77–79, 86, 158, 238
Retina, see Eye
Rheotaxis, 28, 158, 159, 164
*Rhinichthys,* 407
*Rhodeus amarus* (bitterling), 230, 255, 261, 276, 291, 295
*Rhomboidichthys,* see *Bothus*
*Rhombus,* see *Scophthalmus*
*Rhynchocymba nystrani* (eel), 162
Rhythm, of electric organs, 331, 332, 337, 338
of heart, 90, 93–97
locomotory, 66–90, 102, 103
Roach, see *Rutilus*
*Rutilus (Leuciscus) rutilus,* 96, 97, 131, 177, 190, 191, 308, 377

S

Saccopharyngidae (eels), 349, 354
Sachs' organ, see Electric organs
Salinity, effects on fishes, 217, 222, 248, 282
*Salmo* (trout, Atlantic salmon), 4, 5, 148, 161, 169
*S. gairdnerii (S. irideus)* (rainbow trout), 22–27, 43, 103, 374, 376, 400, 423
*S. salar* (Atlantic salmon), 15, 189, 276, 282, 285, 287, 377, 383
*S. trutta* (brown trout), 103, 200, 377, 382, 383, 423, 468, 469, 471, 476
Salmon, see Salmonidae
Atlantic, see *Salmo salar*
chum, see *Oncorhynchus keta*
coho, see *O. kisutch*
Pacific, see *Oncorhynchus* spp.
silver, see *O. kisutch*
sockeye, see *O. nerka*
Salmonidae, 204–206, 224, 294, 317, 413–415
*Salvelinus* (char, trout), 377
*S. alpinus* (char), 282, 294, 295
*S. fontinalis* (brook trout, speckled trout), 137, 161, 274, 405, 407, 414, 423, 472, 475, 476, 478, 480
*S. namaycush* (lake trout), 275, 472, 475
*Sardina pilchardus* (sardine), 133
*Sargus annularis,* 64, 87–90, 95, 177, 180
*Scardinius (Leuciscus) erythrophthalmus* (rudd), 99, 190, 277
Sciaenidae (drumfishes), 177, 282, 315, 316
*Scomber scombrus* (mackerel), 40, 379
*Scophthalmus (Rhombus),* 222, 395
*S. maximus,* see *Bothus maximus*
*S. norvegicus (Phrynorhombus norvegicus),* 379
*S. rhombus,* see *Bothus rhombus*
*Scorpaena scrofa,* 93, 95, 380
*Scyliorhinus* (dogfish), 4, 5, 7, 36, 38, 43, 48, 53, 66, 67, 72, 74, 78, 129, 161, 166, 238
*S. canicula,* 6, 13, 18, 37, 45, 49, 54–57, 71, 73, 80–85, 102
*S. catulus,* 6, 37, 38, 47, 54–57, 71, 73
Sea horse, see *Hippocampus*
*Sebastes marinus,* 380
*Secutor,* 353
Selachii, see Elasmobranchii
*Semotilus atromaculatus* (dace), 141, 181, 407
Senses, correlation centres in brain, 12, 18, 40, 41
Serranidae (sea basses), 349, 354
*Serranus,* 124, 133
Sex linkage, 433–438, 440–442, 445, 460–463

Sex reversal, 438, 439, 446, 492
Sharks, see Elasmobranchii, *Carcharinus*
  repellents, 197
Siluridae (catfish), 281, 283, 294, 315
*Siphostoma typhle,* 190, 381
Skates, see *Raia*
Skin alarm substance, 200, 201
  electric organs, 327, 328, 330
  innervation of, 46–49, 53, 91, 99
  photophores in, 346, 349, 355, 356
  pigments in, 372, 375–383
  sensitivity, 30, 42, 53–58, 64, 65, 67, 69, 73, 76, 396
*Solea solea (vulgaris)* (sole), 47, 379
Sound, perception, 34, 35, 39, 41–43, 178–182, 220, 221, 314–316
  production, 179, 261, 282, 315, 316
  stimuli affecting behavior, 279, 282
*Spheroides maculatus,* 190
  *S. spengleri,* 28
  *S. rubripes,* 139
*Sphyrna* (hammerhead shark), 198
*Spinachia vulgaris* (stickleback), 222, 223
Spinal cord, 16, 36, 38, 42, 46–90, 97–99, 102, 327, 330, 337, 340
*Spinax,* see *Etmopterus*
Squalidae, 346, 355
*Squalius cephalus* (chub), 4, 6, 36, 422, 423
*Squalus acanthias (Acanthias vulgaris)* (spiny dogfish), 7, 13, 48, 56, 74, 80–83, 85, 137, 165, 397
Stargazers, see *Astroscopus, Uranoscopus,* 125, 148
*Steatogenys elegans,* 328
*Stenotomus chrysops (S. versicolor),* 379
Sternoptychidae, 349
Sticklebacks, see *Gasterosteidae, Gasterosteus, Pygosteus, Spinachia*
Stomiatoidea (lantern fishes), 317, 349, 356
Strychnine, 49, 50
Sunfish, see Centrarchidae, *Eupomotis, Lepomis*
Swimbladder, 305–318
  nervous control of, 92, 312
Swordtail, see *Xiphophorus*
Symbranchidae, 147
Sympathetic nervous system, see Autonomic nervous system
*Syngnathus acus* (pipefish), 133, 381

T

Taste, gustatory nerves, 4, 12, 16, 42
  perception, 6, 42, 196, 197, 221
  receptors, 195–197
  stimuli affecting behavior, 282
*Tautoga onitis,* 136
Taxes, see Reflexes, orienting
Telencephalon (forebrain), 3–12, 288–290, 404
  basal ganglia (corpora striata), 4, 5, 289
Teleostei, 3–5, 7, 16, 17, 28, 32, 35, 38–40, 43, 44, 47, 57–65, 74–76, 92, 103, 125, 123–128, 130, 131, 136, 141, 143, 148, 149, 157, 190, 288, 290, 291, 305–317, 323, 324, 363, 374, 376–381, 397, 453
Temperature, and breeding, 245, 248, 250, 274, 275
  effects on fishes, 404–409, 413, 414, 468–471, 475, 476
  and electric discharge, 332, 336
  perception, 163, 167, 168, 222, 223
Tench, see *Tinca tinca*
Tetrapyrroles, 367
Thalamus, see Diencephalon
*Thunnus thynnus* (tuna), 15, 40, 204
Thyroid, gland, thyroxine, 288, 393, 400
  tumors, 467, 478–486, 492
*Tilapia,* 242, 247, 249, 288
  *T. macrocephala,* 274, 278, 285, 289–291, 294, 295
  *T. mossambica,* 257, 279, 280
*Tinca tinca* (tench), 33, 36, 57, 67, 71, 74, 78, 94, 96, 97, 131, 137–139, 148, 177, 193, 308, 313
*Torpedo,* 7, 48, 72, 149, 156, 165, 323–326, 328, 329, 332, 334–339
  *T. marmorata,* 37, 330, 331
  *T. nobiliana (T. occidentalis),* 330, 331, 338
Touch (and current), 16, 39, 42, 55–65, 71–74, 76, 77, 79–81, 84, 239, 255, 261, 279, 282, 283
*Toxotes jaculator* (archer fish), 132
*Trachinus draco* (weever), 380
Transplants, 473–475

*Trichogaster leeri,* 176
  *T. trichopterus,* 176
*Trichopsis vittatus* (gourami), 282
*Trigla gurnardus* (gurnard), 7, 19, 196, 315, 380
Trout, see Salmonidae
  brook, see *Salvelinus fontinalis*
  brown, see *Salmo trutta*
  lake, see *Salvelinus namaycush*
  rainbow, see *Salmo gairdnerii (S. irideus)*
*Trygon,* see *Dasyatis*
Tuna, see *Thunnus*
*Typhlobagrus kronei* (blind catfish), 467
*Typhlogobius californiensis* (blind goby), 278, 279
*Typhlonarke* (blind torpedo), 339

U

*Umbra limi* (mudminnow), 140, 141, 176, 180
  *U. pygmaea,* 176
*Uranoscopus* (stargazer), 64, 97, 128, 312
Urethane, 17, 77
Urinogenital system, nervous control of, 93

V

Vacuum activity, see Behavior
Vascular system, arteries to swimbladder, 307
  nervous control of, 93–96
Vesicles of Savi, see Acousticolateralis system
Vision, see Eye, Light
Vitamins, A, 383
  B, 370

W

Weberian ossicles, see Acousticolateralis system
Wrasses, see Labridae

X

*Xiphophorus* (platyfish, swordtail), 140, 149, 287, 292, 293, 374, 440, 443, 455, 479
*X. couchianus,* 481
*X. helleri,* 285, 286, 289, 377, 440–447, 454, 456–458, 462–466, 473, 481, 486–491, 493
*X. maculatus (Platypoecilus maculatus)* (platyfish), 289, 290, 377, 434–437, 439–446, 448–466, 473–475, 479, 481–491, 493
*X. montezumae,* 445, 453, 466, 474, 479–484
*X. pygmaeus,* 481
*X. variatus (Platypoecilus variatus),* 287, 445, 466, 481
*X. xiphidium,* 445, 481

Y

*Yarella,* 349
Yohimbine, 99

Z

*Zenion,* 144
*Zeus faber* (John Dory), 191, 308
*Zoarces viviparus* (viviparous blenny, eelpout), 190, 222, 223, 381

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